

FIG.1

FIG. 2

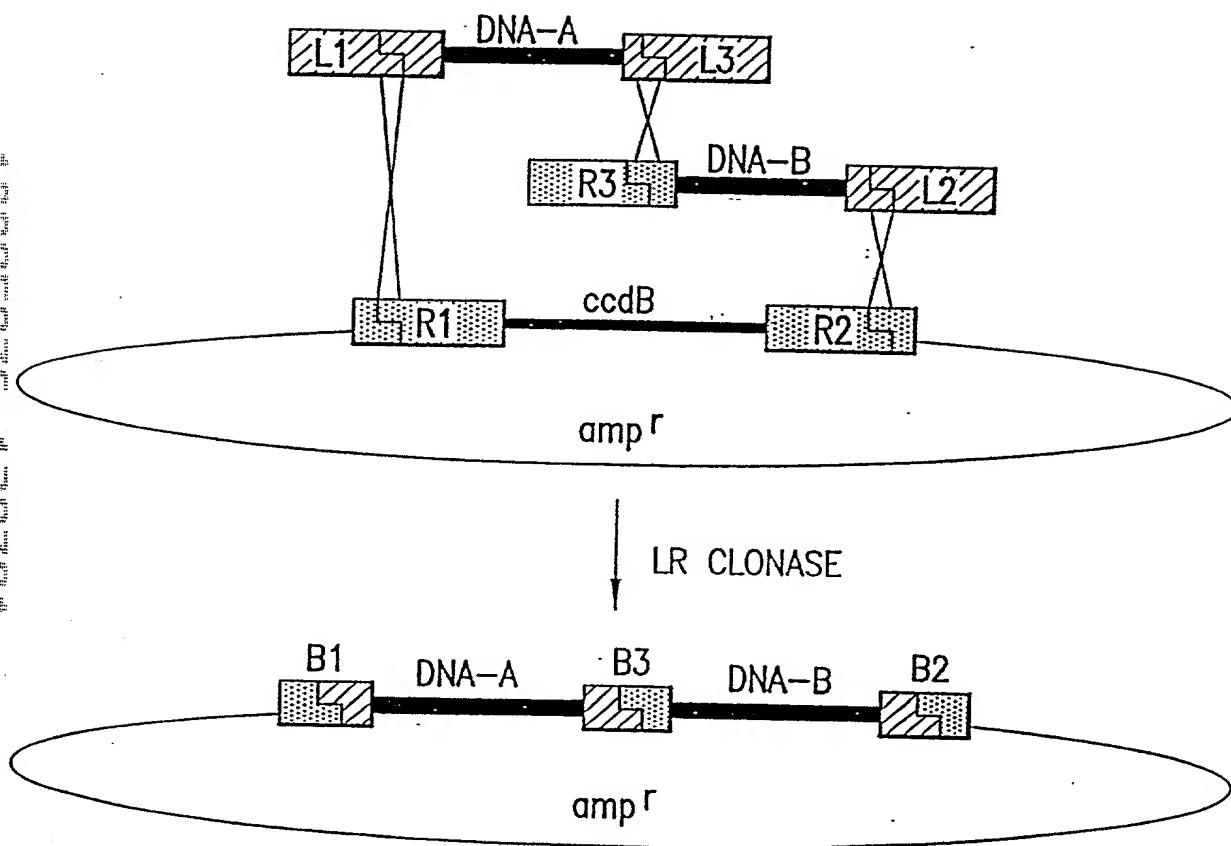


FIG.2

FIG. 3

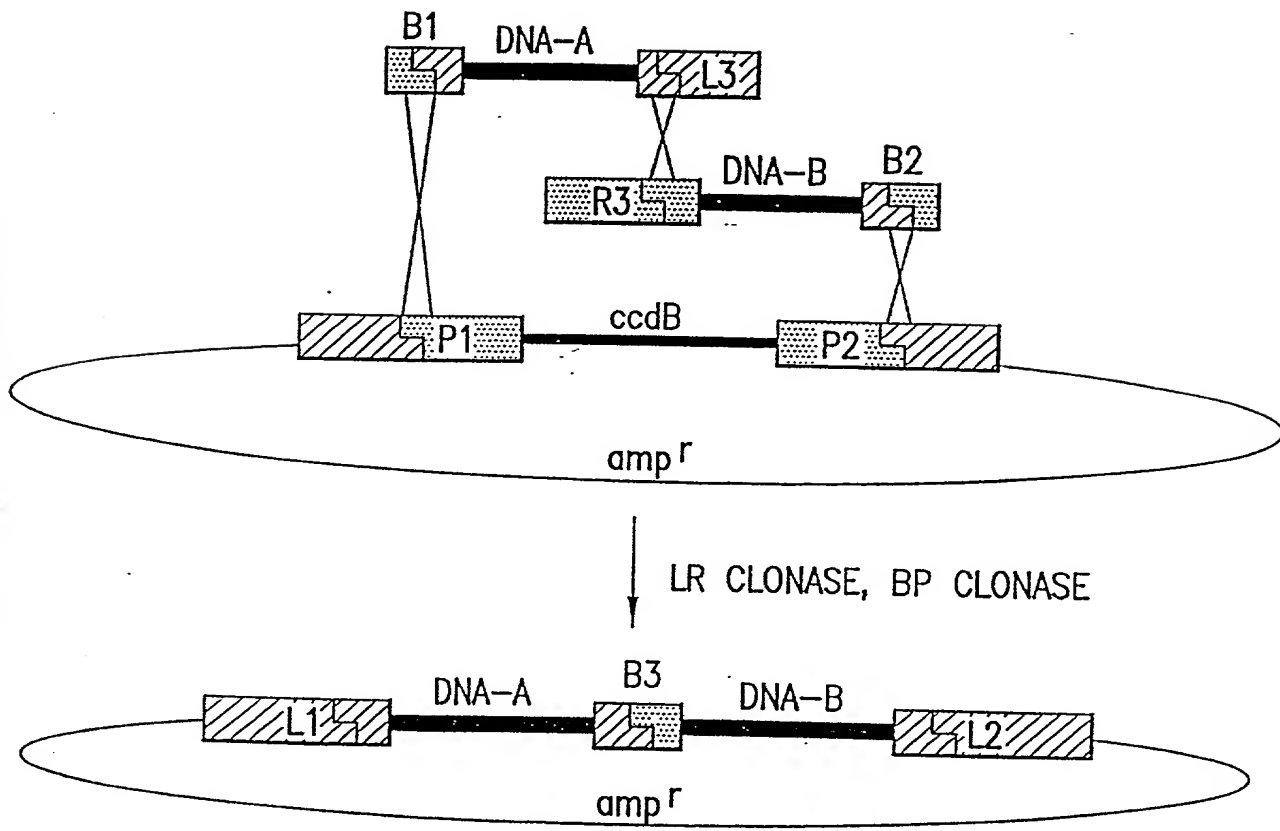


FIG.3

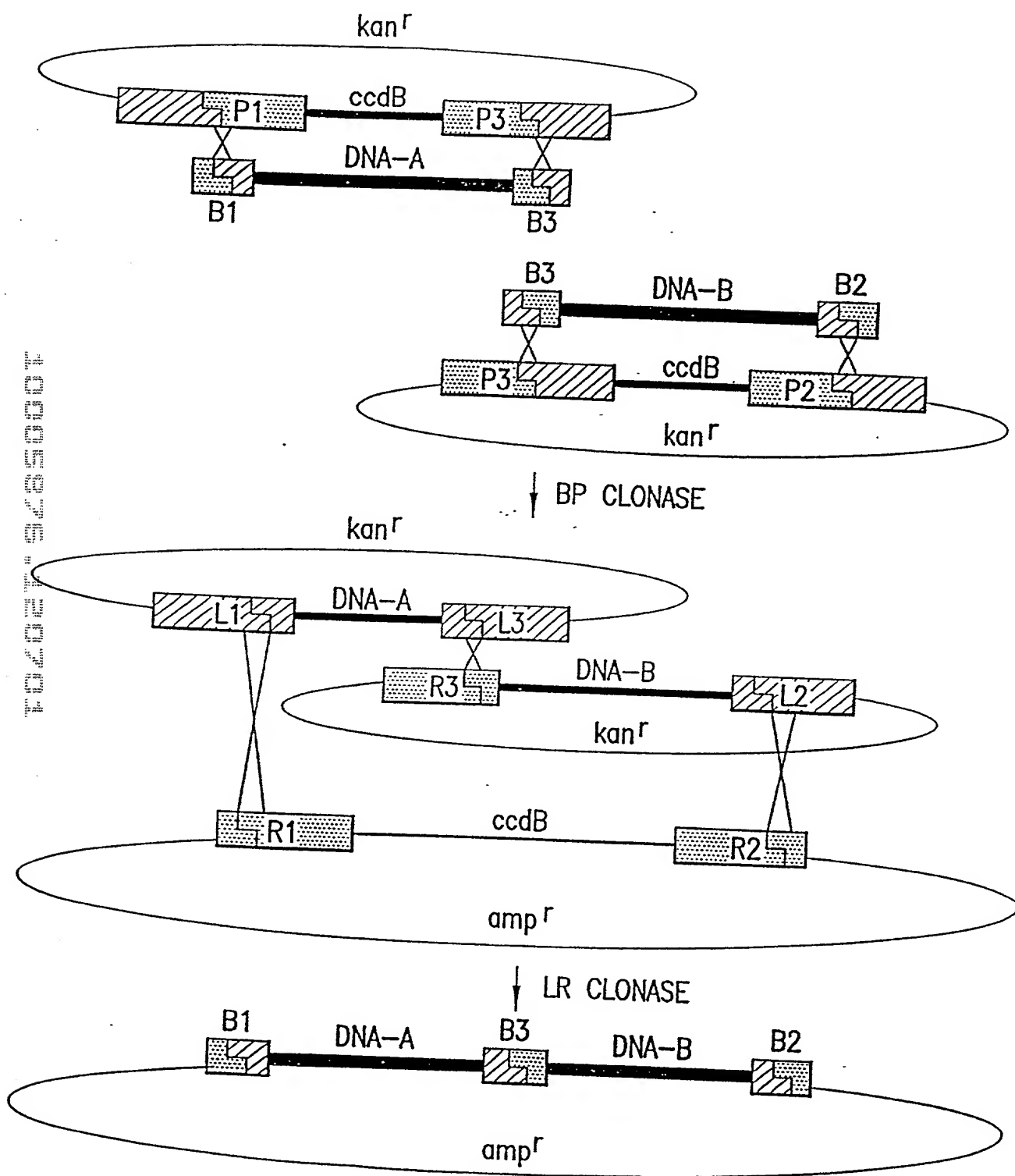


FIG.4

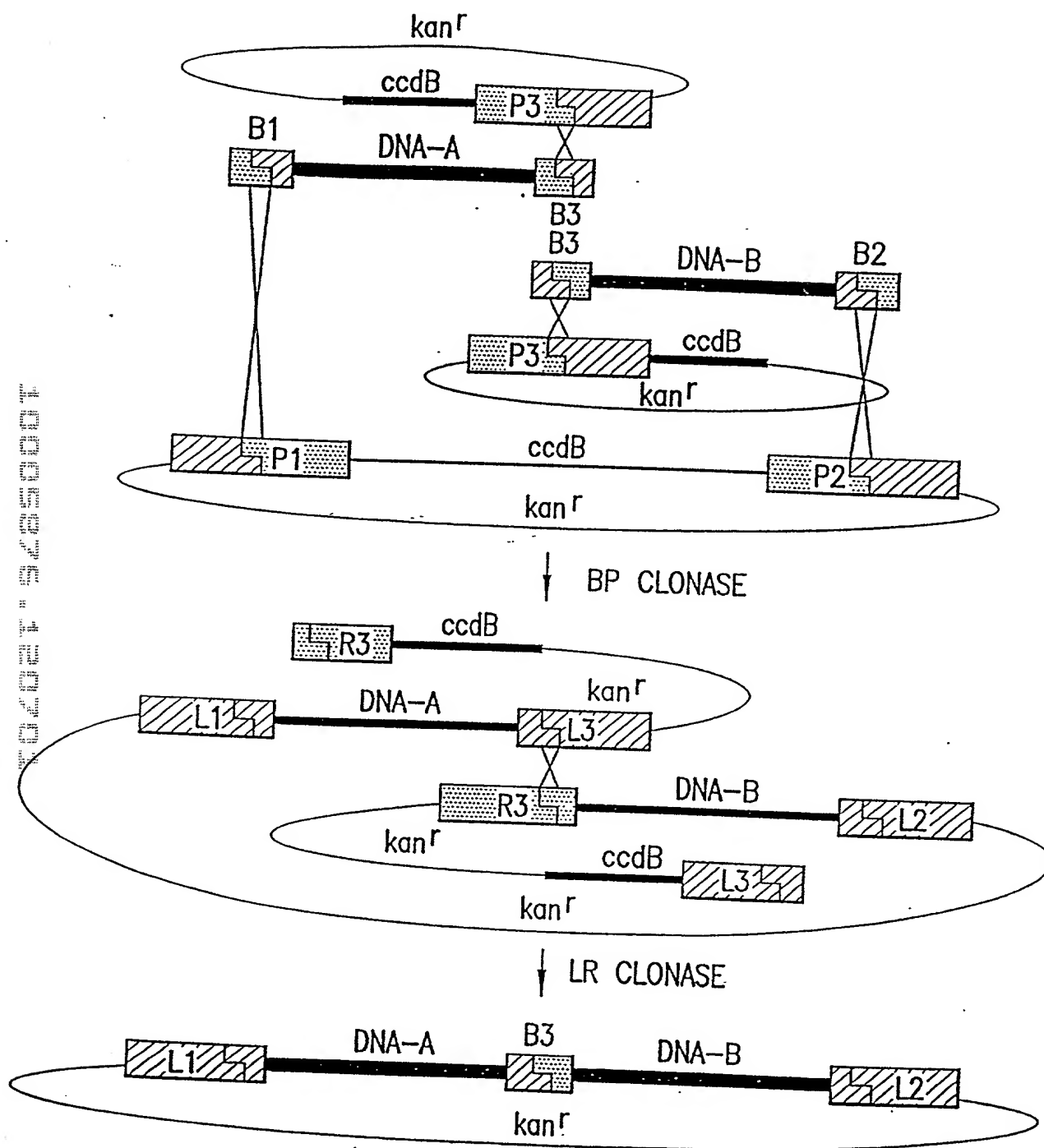


FIG.5

FIG. 6

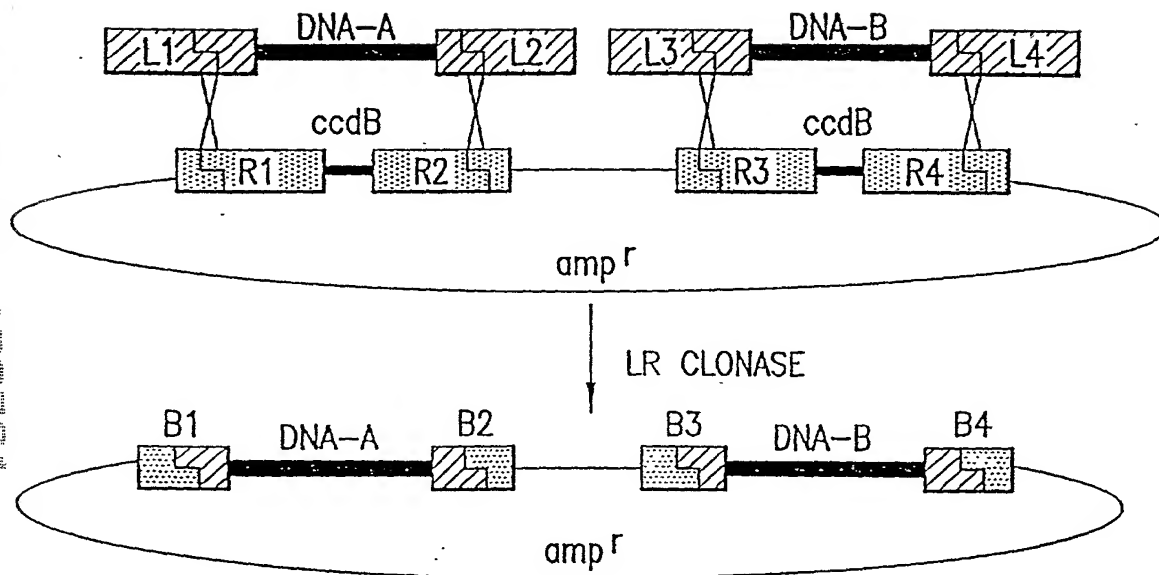


FIG.6

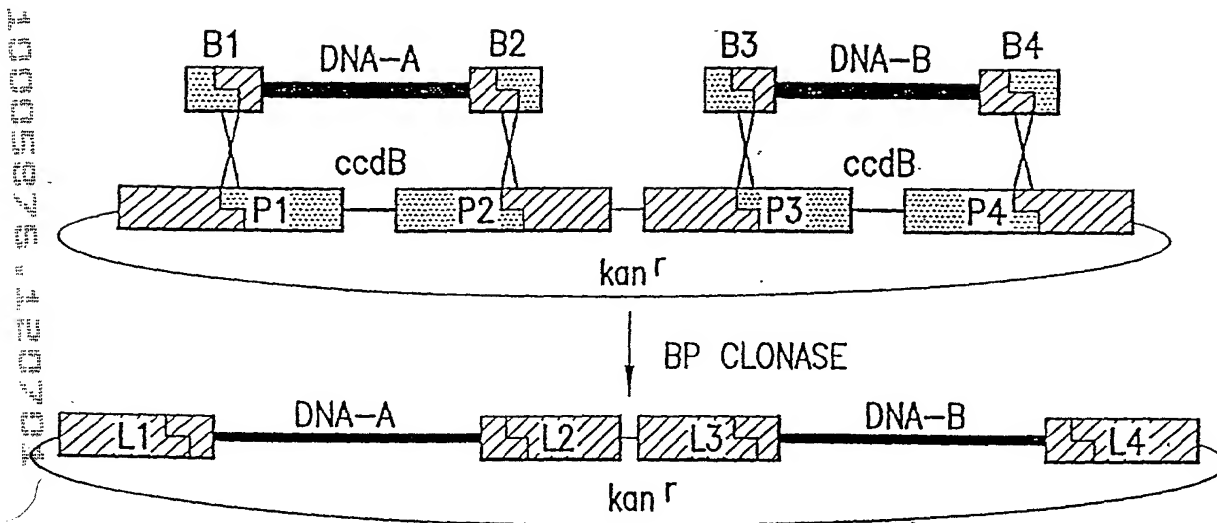


FIG.7

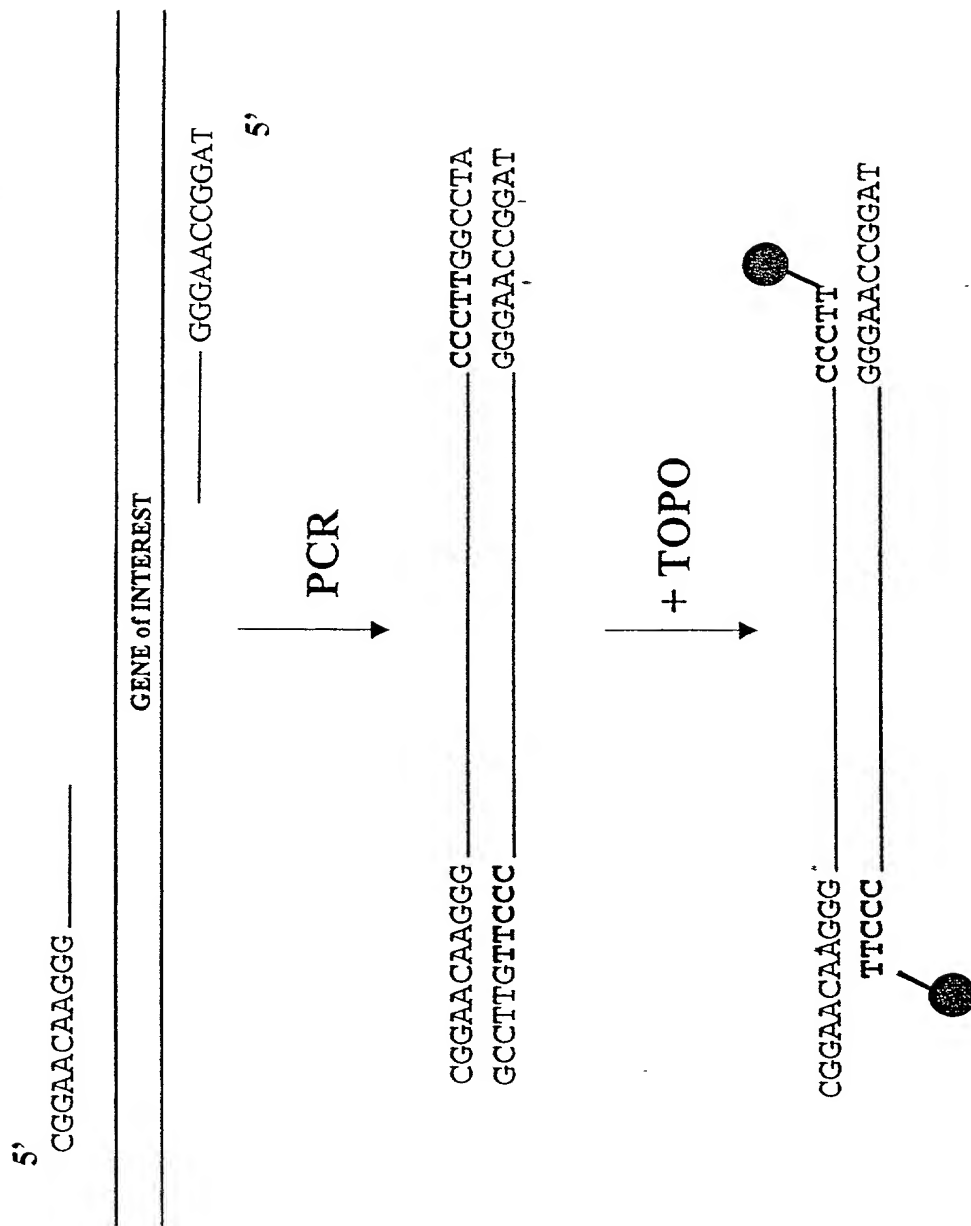


FIGURE 8A

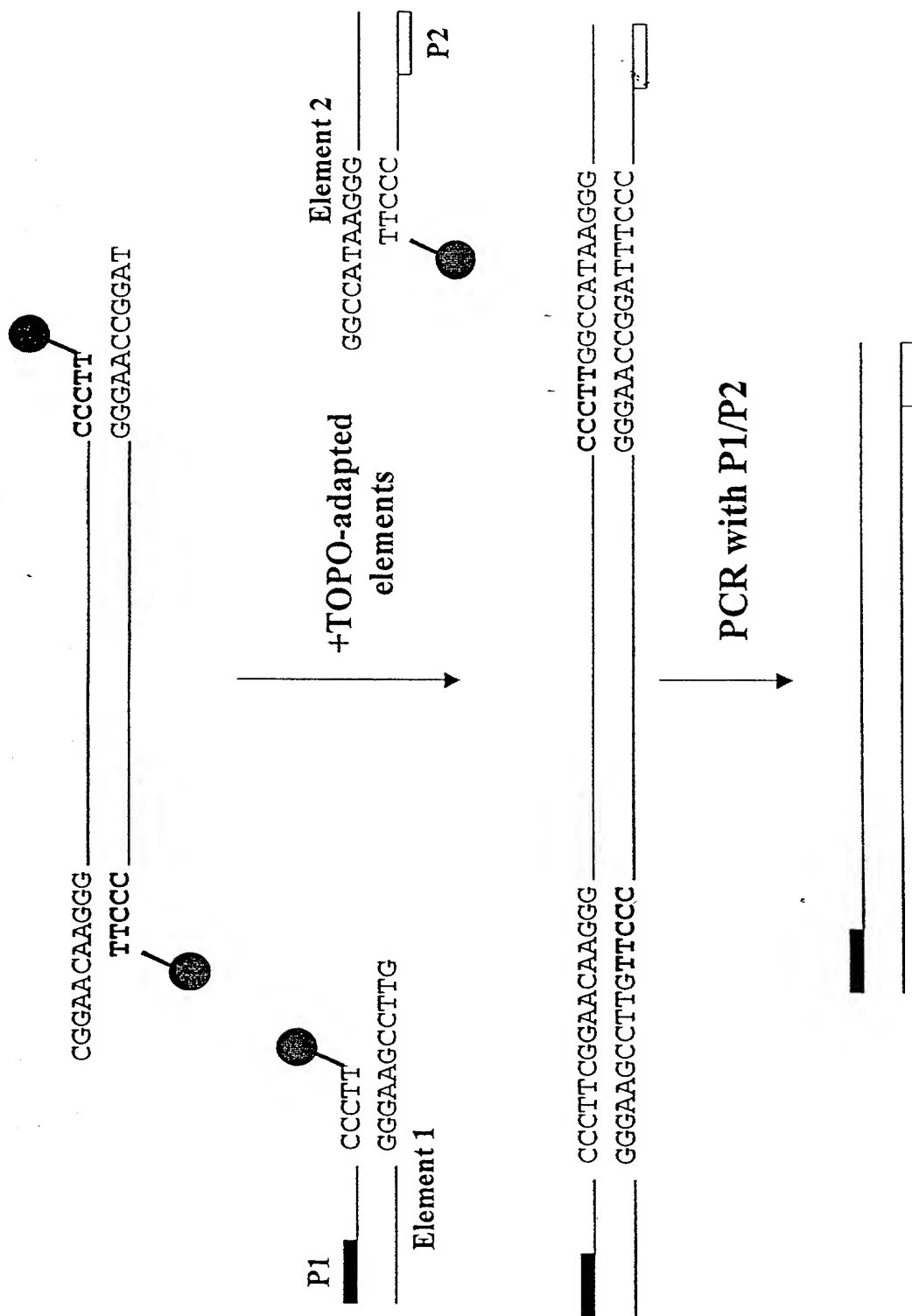


FIGURE 8B

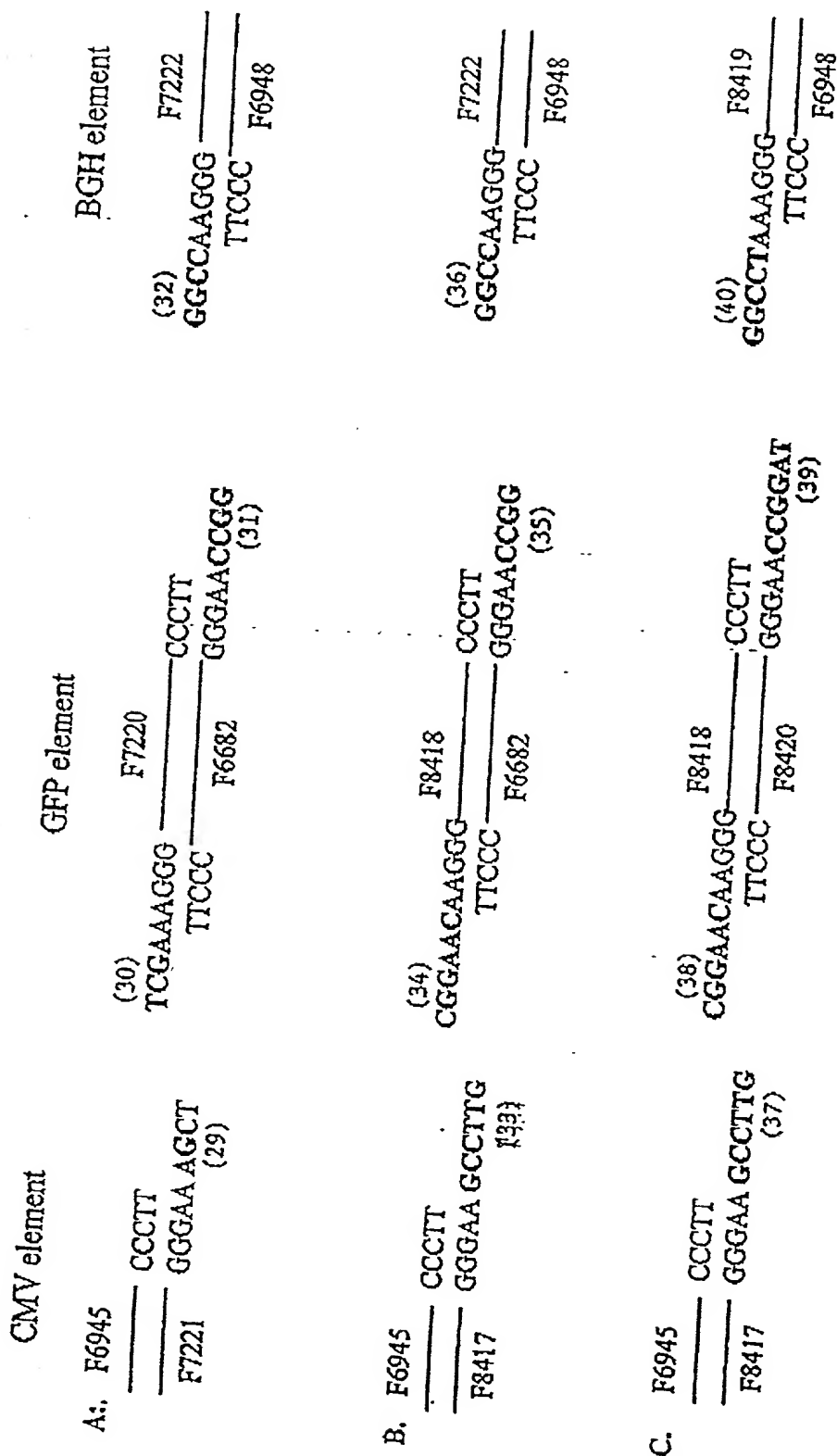


Figure 9A-C

Table 1

Primer name	F#	Sequence (5' → 3')	SEQ ID NO:
MTH1	10779	TATGTATCATACATACGATTAGGT	1
MTH2	10780	ACCGCTCTCCCGCGGT	2
GAL4r2	12667	GTCCGAAGGGGGGATACAGTCAACTGTCTTG	3
MTH5	12505	TTGGCCAAGGTATCTAGAAGCTTCTGCAGACGCGT	4
VP16r2	12668	GTCCGAAGGGGGGATCTGCTCAATTCGAAG	5
SV40pA	12018	GGCCAAAGGGAACTGTTATTCAGCTTATAATG	6
SV40pA	561	CTCTGACTTGAGCGTCGATTT	7
p53r2	12669	CGGAACAAGGGGAATCCCTGTCCACCGAGACC	8
SVT2	12670	CGGAACAAGGGGAATCCCGGGGATCTGGAATTC	9
CMVr2	7221	TCGAAAGGTCGAGGTCGACCTGCAGCTG	10
CMVr	6945	AATTCACATTGATTATTCAGTAGTTA	11
GFP-XhoI	7220	TCGAAAGGTAATGGCCAGCAAGGAGAAG	12
GFP-Notr	6682	GGCCAAGGGTTGTAGAGCTCATCCAT	13
BGHr2	7222	GGCCAAGGGTCTGAATGGGGCCGCATAGT	14
BGHr	6948	AAGCCATAGAGCCCGGGCCA	15
CMVr3	8417	GTCCGAAGGGTCGAGGTCGACCTGCAGCTG	16
GFPi3	8418	CGGAACAAGGGATGGCCAGCAAGGAGAAG	17
GFPi3	8420	TAGGCCAAGGGTTGTAGAGCTCATCCATGC	18
BGHr3	8419	GGCCTAAAGGGTGAATGGGGCCGCATAGT	19
T7top	9304	GAAGGAGTAATACGACTCAGTATAGGAGCCACCATTGGCCCTTCGGAAC	20
T7bottom	9305	GTCCGAAGGGGCCCATGGTGGCTCCCTATAGTGTGATTAATCTCTCTTC	21
T7amp	9306	GAAGGAGTAATACGACTCAGT	22
T3bp	9661	GGCCTAAAGGGTCCCTTTAGTGAGGGTTAATTGCGCGC	23
T3bottom	9662	GGCGGCAATTAAACCTCAGTAAAGGAGCCCTTAGGCGC	24
lacZi2	10632	CGGAACAAGGGATGATAGATCCCGTGGTTTACA	25
lacZi1k2	10770	TAGGCCAAGGGGACCATTTTCAATCCGCAAGT	26
lacZ2k2	10771	TAGGCCAAGGGGAGGAGCACTTCAACCGCTTGCCA	27
lacZ3k2	10772	TAGGCCAAGGGTTTGACACCAGACCAACTGGTA	28

Figure 9D

A.

Sample #	GAL4+pA	VP16+pA	pGene/lacZ	GAL4+p53+pA	VP16+1+pA	p53-VP16
1			0.26 ug	p 0.37 ug	p 0.37 ug	
2			0.4 ug	p 0.3 ug	p 0.3 ug	
3			0.4 ug			p 0.6 ug
4			0.4 ug	10.3 ug	10.3 ug	
5		10.3 ug	0.4 ug	10.3 ug		
6	10.3 ug		0.4 ug		10.3 ug	
7			0.4 ug	4.5 ul PCR	4.5 ul PCR	
8		4.5 ul PCR	0.4 ug	4.5 ul PCR		
9	4.5 ul PCR		0.4 ug		4.5 ul PCR	

B.

Sample #	LacZ activity
1	240000
2	140000
3	1800000
4	1400000
5	54000
6	80000
7	320000
8	12000
9	42000

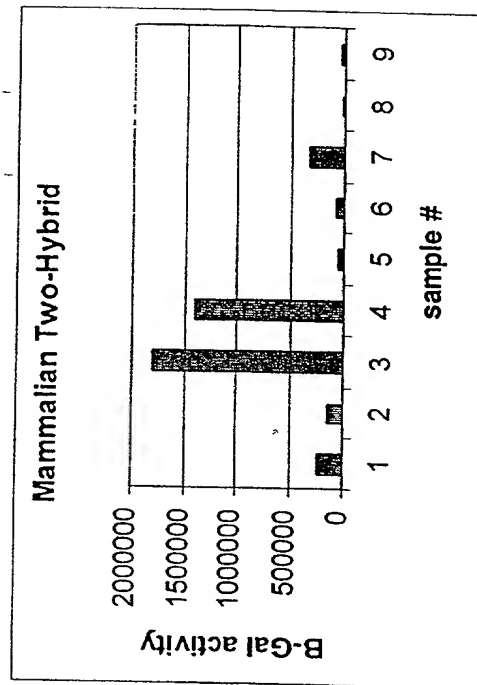


FIGURE 10

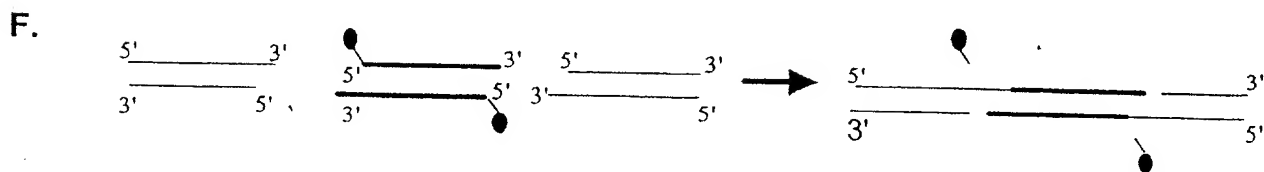
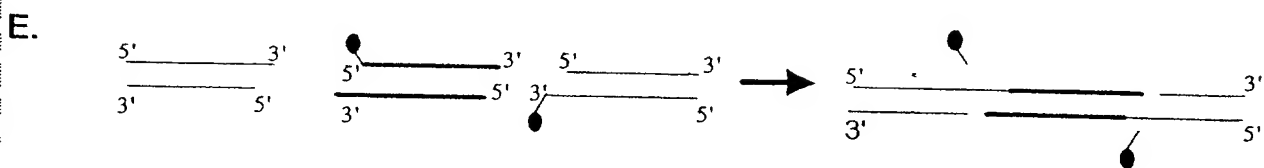
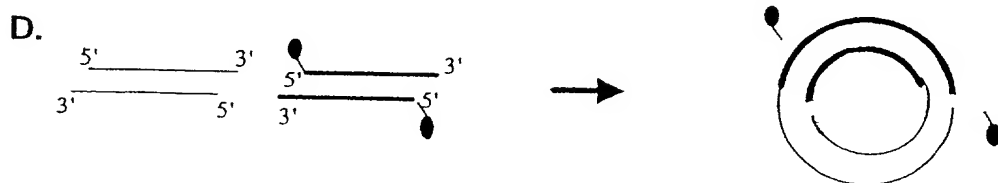
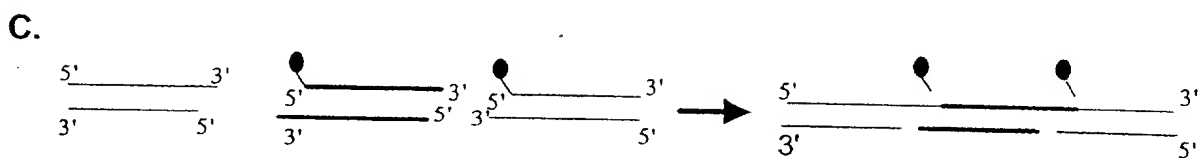
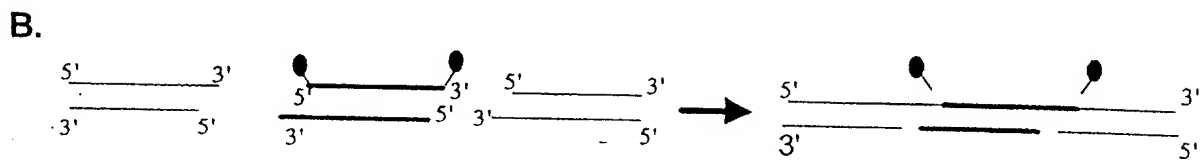
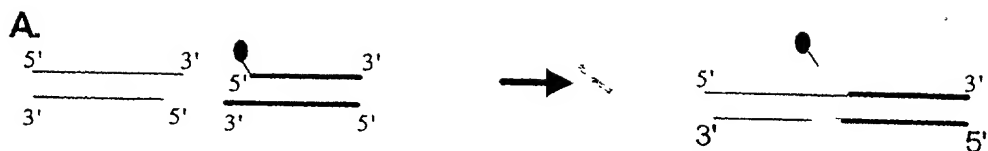
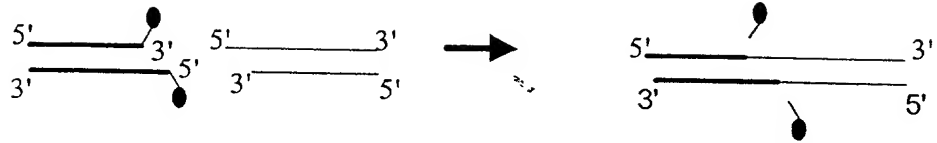
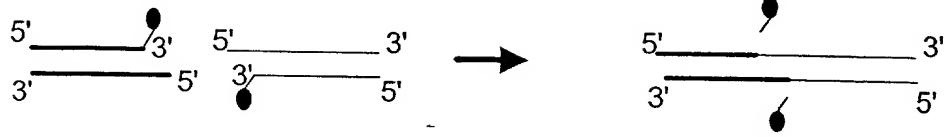


FIGURE 11

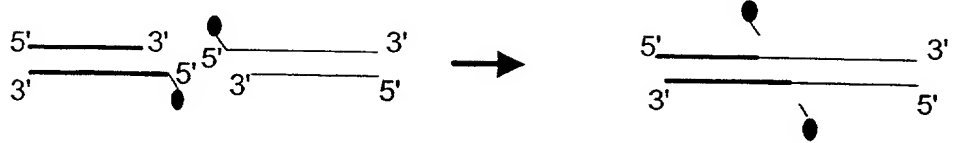
A.



B.



C.



D.

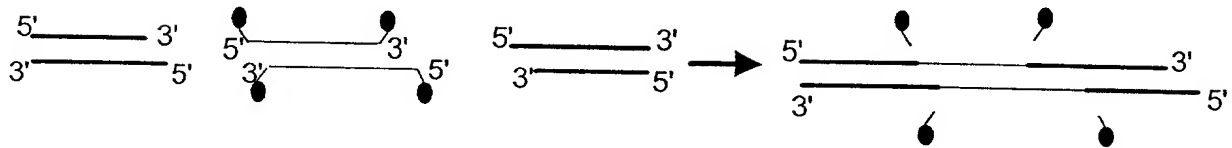


FIGURE 12

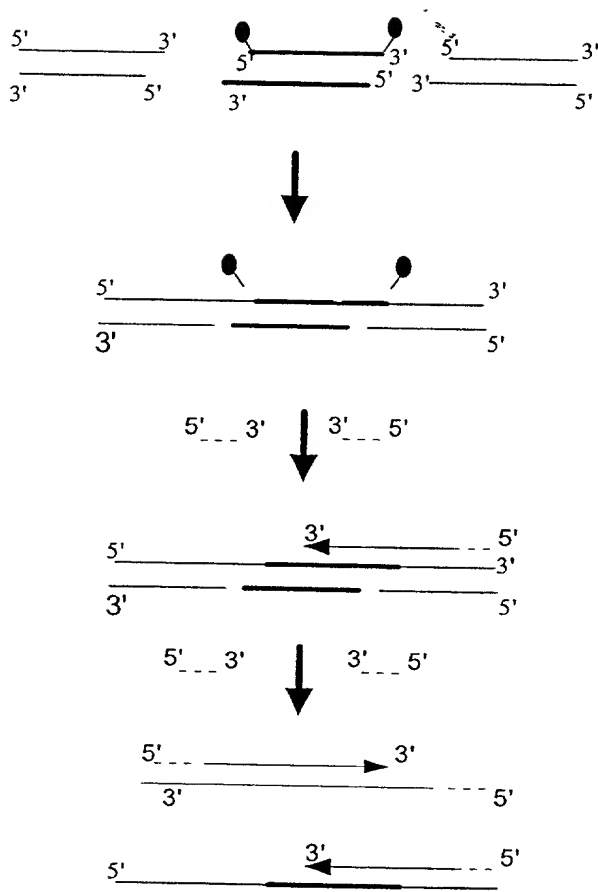
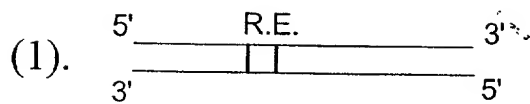


FIGURE 13

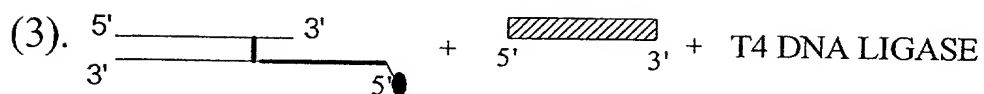
FIGURE 14



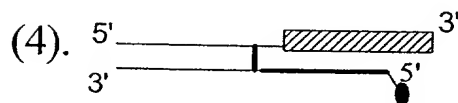
A
↓
RESTRICTION
ENZYME
DIGESTION



B
↓



C
↓



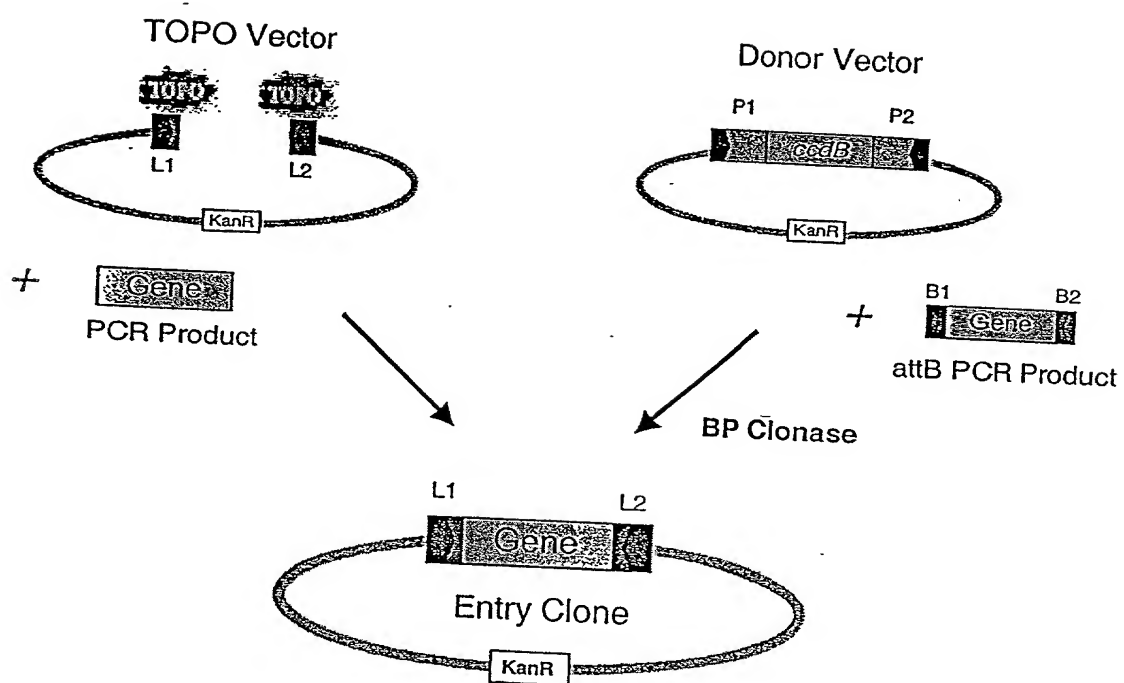


FIGURE 16

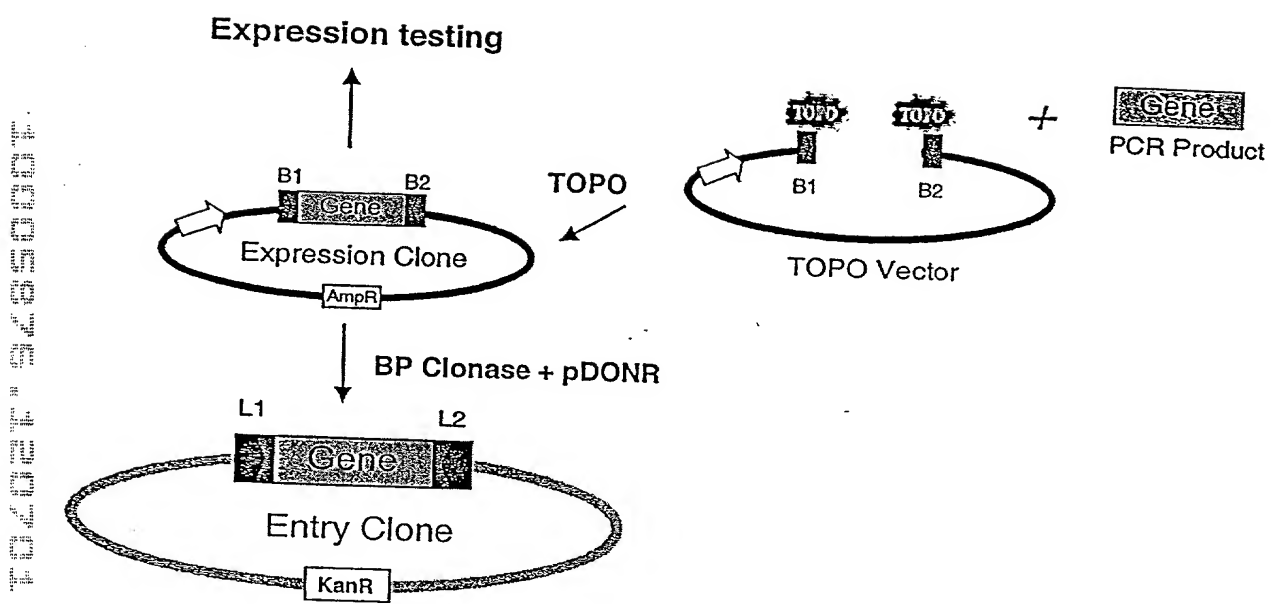


FIGURE 17

MCS for pcDNAGW-DT(sc) and pENTR-DT(sc)

L Y K K A G S A A A G R A D P A F L Y K V
 ...TTG TAC AAA AAA GCA GGC TCC GCG GCC GCC GTA CTC GAG AAA GGG CGC GCC GAC CCA GCT TTC TTG TAC AAA GTG
BsrGI *NotI* *XhoI* *AscI* *BsrGI*


FIGURE 18

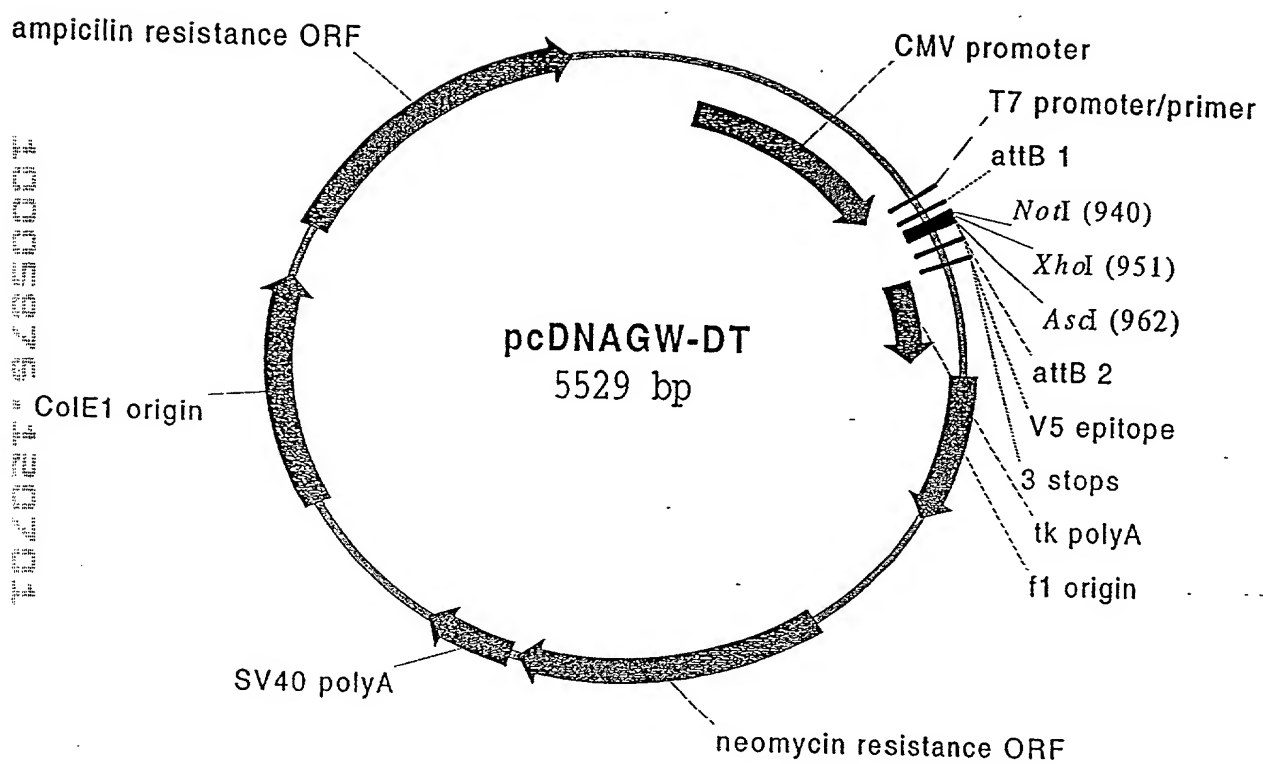


FIGURE 19

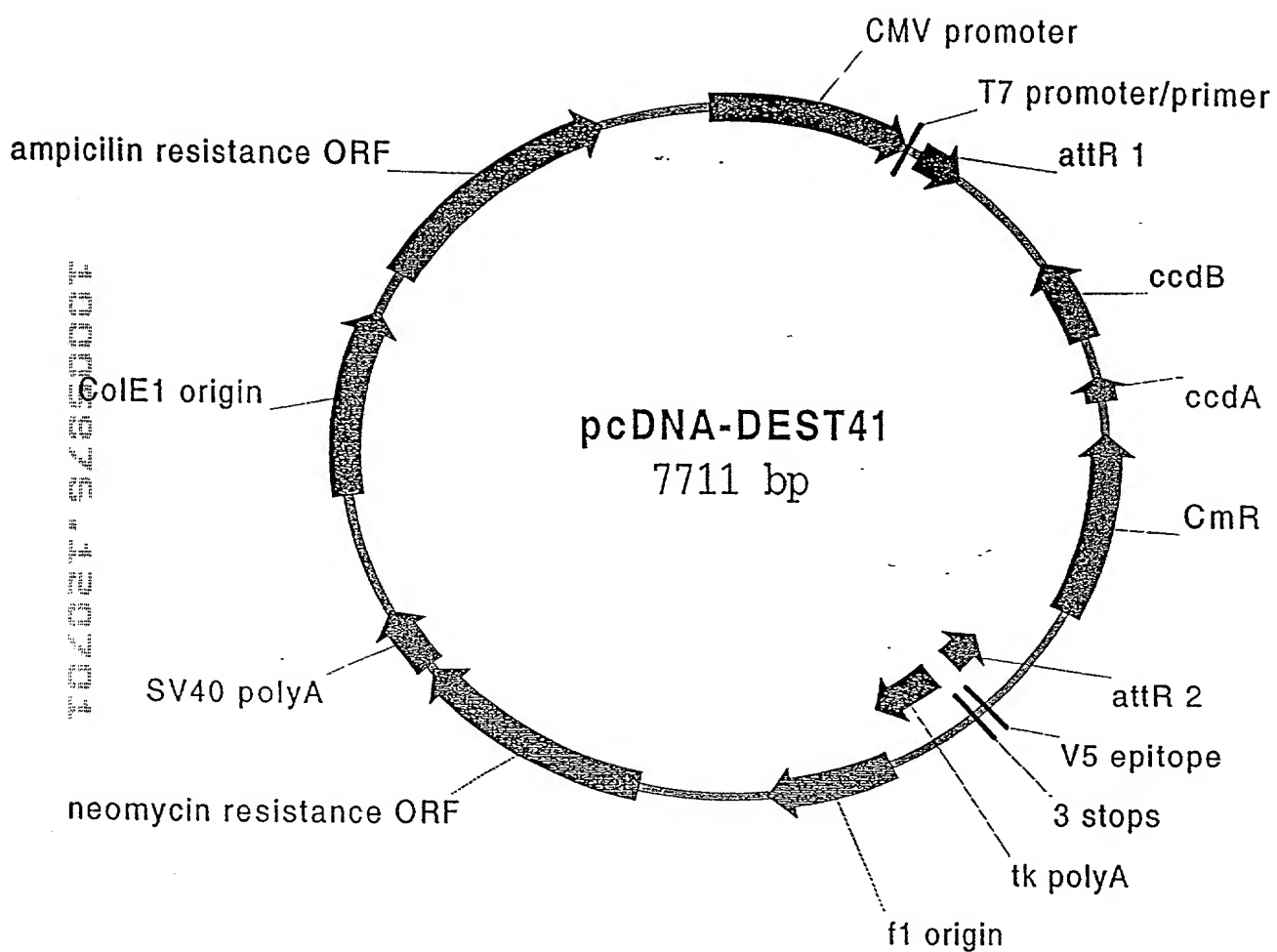


FIGURE 20

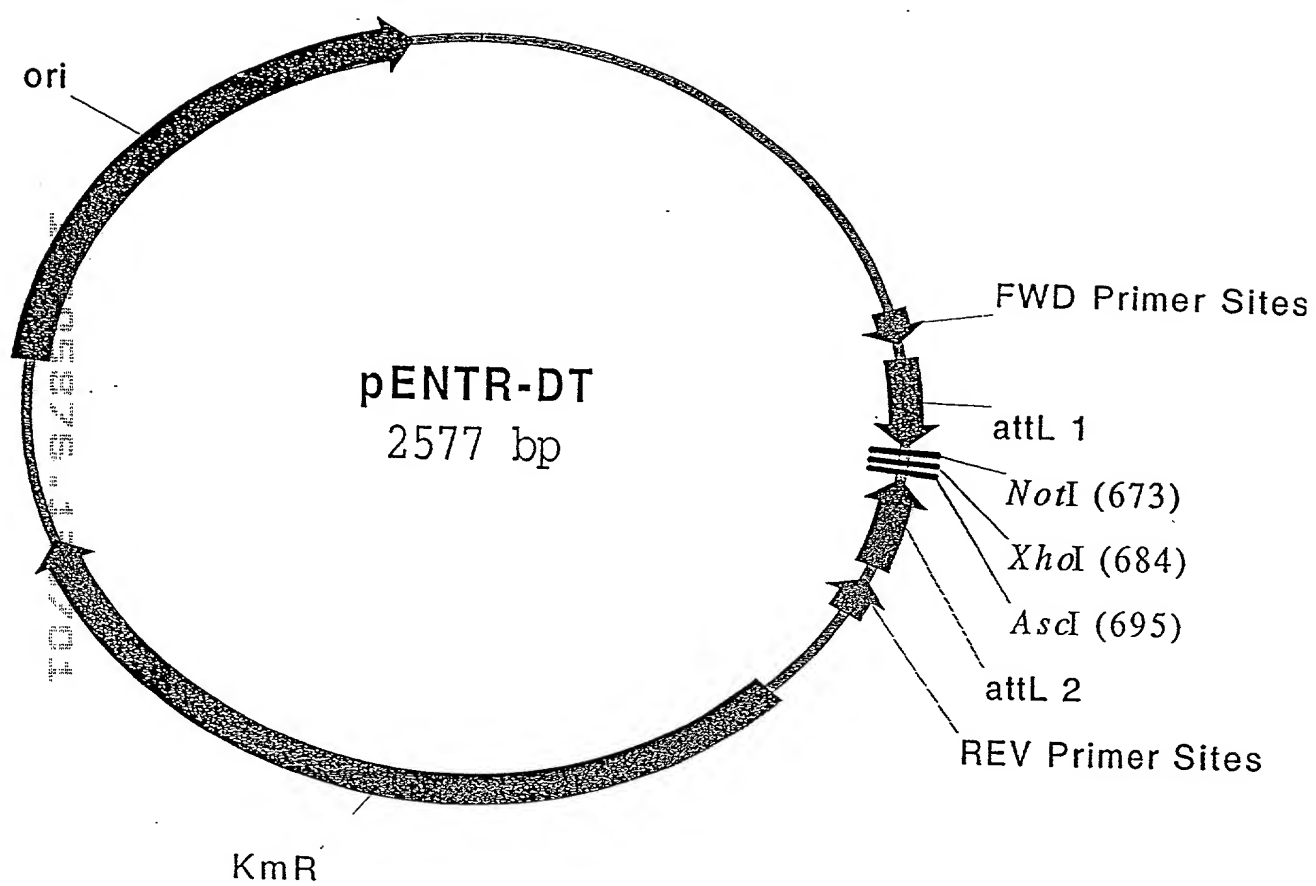


FIGURE 21

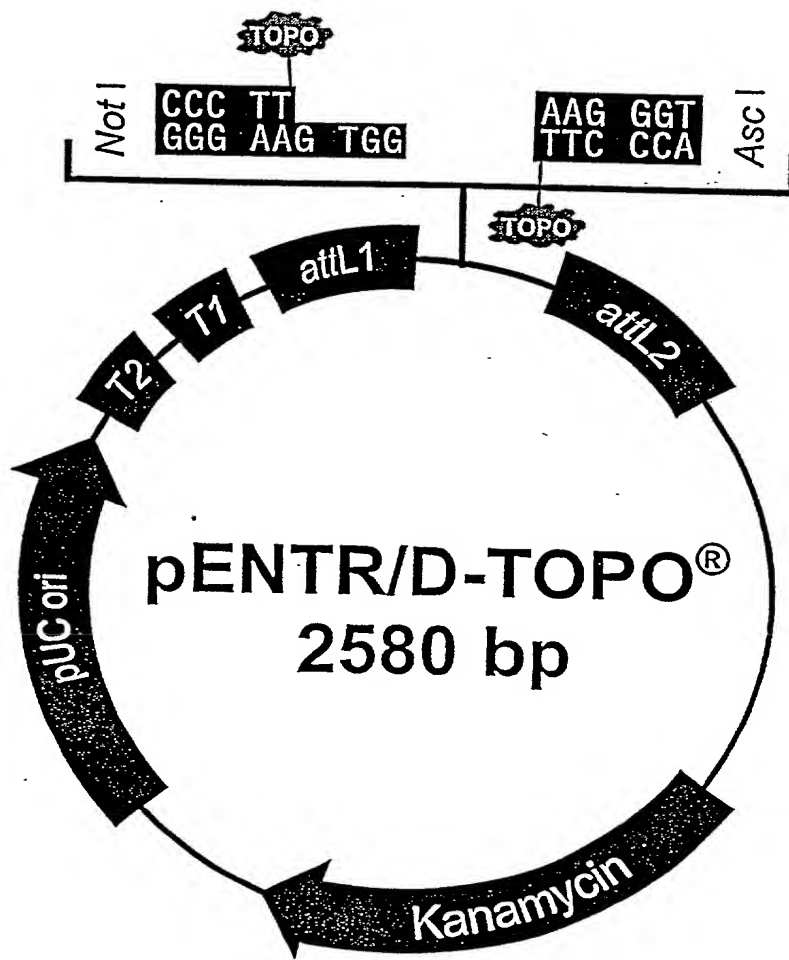


FIGURE 22A


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121  gcgcccgaata  cgcaaaccgc  ctctccccgc  gcgttgggcg  attcattaat  gcagctggca
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241  tagccaggaa  gagttttag  aaacgcaaaa  aggccatccg  tcaggatggc  cttctgctta
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361  acaacgttca  aatccgctcc  cggcggatgt  gtccactca  ggagagcggt  caccgacaaa
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601  ctggtcgttg  caacaaattg  atgagcaatg  cttttttata  atgccaactt  tgtacaaaaa
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841  gagtcgtatt  acatgggtcat  agctgtttcc  tggcagctct  ggcccgtgtc  tcaaaatctc
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2221  agacgatagt  taccggataa  ggcgagcgcg  tcgggctgaa  cgggggggtt  gtgcacacag
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2341  agcggcacgc  ttcccgaagg  gagaaaggcg  gacaggtatc  cggtaagcgg  cagggtcgga
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2461  gggtttcgcc  acctctgact  tgagcgtcga  tttttgtgat  gctcgtcagg  ggggaggagc
2521  ctatggaaaa  acgccagcaa  cgcggccttt  ttacggttcc  tggccttttg  ctggcctttt
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FIGURE 22B

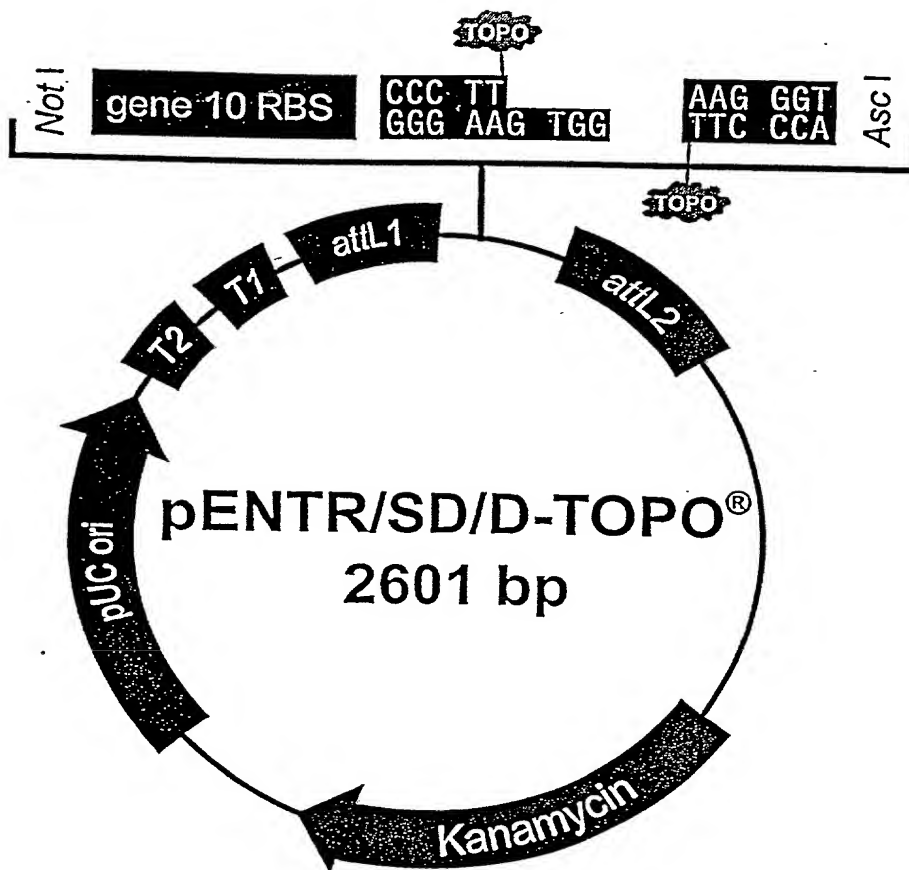


FIGURE 23A

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121 gcgcccataa cgcaaaccgc ctctcccgc gcgttggccg attcattaat gcagctggca
181 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
241 tagccaggaa gagttttag aaacgcaaaa aggccatccg tcaggatggc cttctgctta
301 gtttgatgcc tggcagttta tggcgggcbt cctgcccgc accctccggg ccgttgcttc
361 acaacgttca aatccgctcc cggcggttt gtccactca ggagagcgtt caccgacaaa
421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgctg
481 gcagttccct actctcgcgt taacgctagc atggatgttt tccagtcac gacgttgtaa
541 aacgacggcc agtcttaagc tcgggcccc aataatgatt ttattttgac tgatagtac
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721 tgggcgcgcc gaccagctt tctgtacaa agttggcatt ataagaaagc attgcttato
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1561 accggattca gtcgtcactc atggtgattt ctcacttgat aaccttattt ttgacgaggg
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2041 agagctacca actctttttc cgaaggtaac tggcttcagc agagcgcaga taccaaatac
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2161 atacctcgct ctgctaatec tgttaccagt ggctgctgcc agtggcgata agtcgtgtct
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FIGURE 23B

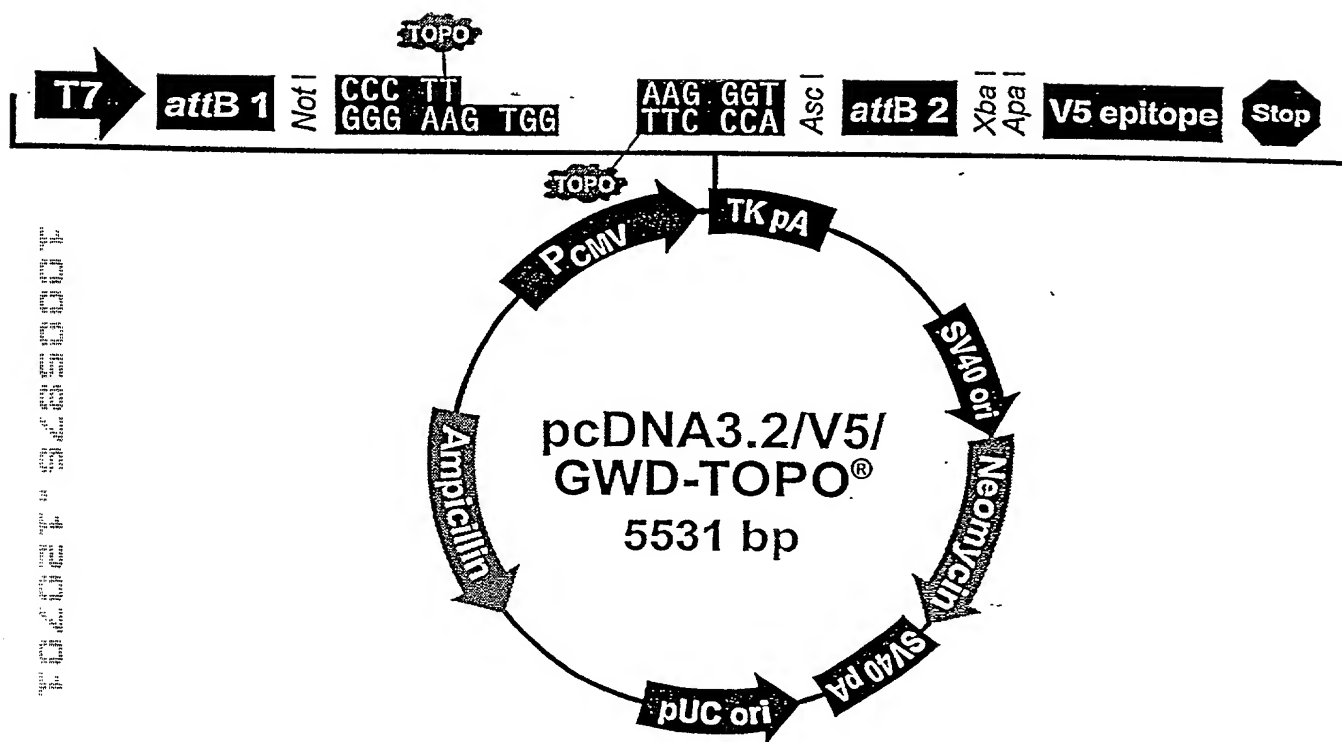


FIGURE 24A

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181	ttagggttag	gcgttttgcg	ctgcttcgcg	atgtacgggc	cagatatacg	cgttgacatt
241	gattattgac	tagttattaa	tagtaatcaa	ttacggggtc	attagttcat	agcccatata
301	tggagttccg	cgttacataa	cttacggtaa	atggccccgc	tggctgaccg	cccaacgacc
361	cccgccatt	gacgtcaata	atgacgtatg	ttcccatagt	aacgccaata	gggactttcc
421	attgacgtca	atgggtggac	tatttacggg	aaactgcccc	cttggcagta	catcaagtgt
481	atcatatgcc	aagtacgccc	cctattgacg	tcaatgacgg	taaatggccc	gcctggcatt
541	atgcccagta	catgacctta	tgggactttc	ctacttgcca	gtacatctac	gtattagtca
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661	actcacgggg	atttccaagt	ctccacccca	ttgacgtcaa	tgggagtttg	ttttggcacc
721	aaaatcaacg	ggactttcca	aaatgtcgta	acaactccgc	cccatgtacg	caaatggggcg
781	gtaggcgtgt	acggtgggag	gtctatataa	gcagagctct	ctggctaact	agagaaccca
841	ctgcttactg	gcttatcgaa	attaatacga	ctcactatag	ggagacccaa	gctggctagt
901	taagctatca	acaagtttgt	acaaaaaagc	aggctccgcg	gccgcccctt	caccatgnnn
961	nnnnnnaagg	gtggggcgcg	cgacccagct	ttcttgtaca	aagtggttga	tctagagggc
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1261	cccattgggg	ccaatacgcc	cgggttttctt	ccttttcccc	acccaccccc	ccaagttcgg
1321	gtgaaggccc	agggctcgca	gccaacgtcg	ggcgggcagg	ccctgccata	gcagatctgc
1381	gcagctgggg	ctctaggggg	tatccccacg	cgcctgttag	cggcgcatga	agcgcggcgg
1441	gtgtggtggg	tacgcgcagc	gtgaccgcta	cacttgccag	cgccctagcg	cccgtctcct
1501	tcgcttttctt	cccttccttt	ctcgccacgt	tcgcccggctt	tccccgtcaa	gctctaaatc
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1621	attaggggtga	tgggtcacgt	agtggggccat	cgcctgtata	gacgggtttt	cgccctttga
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1741	ctatctcggt	ctattctttt	gatttataag	ggattttggg	gatttcggcc	tattggttaa
1801	gcaatgagtc	gatttaacaa	tatctccacg	cgaattaatt	ctgtggaatg	tgtgtcagtt
1861	aggggtgtgga	aagtccccag	gtccccagc	aggcagaagt	atgcaaagca	tgcactctcaa
1921	ttagtcagca	accagggtgtg	gaaagtcccc	aggctcccca	gcaggcagaa	gtatgcaaag
1981	catgcatctc	aattagtcag	caaccatagt	cccggcccta	actccgcccc	tcccgcccct
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FIGURE 24B

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3301	ctagttgtgg	tttgtccaaa	ctcatcaatg	tatcttatca	tgtctgtata	ccgtcgacct
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3481	gagtgagcta	actcacatta	attgcgttgc	gctcactgcc	cgctttccag	tcgggaaacc
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4621	ccgtcgtgta	gataactacg	atacgggagg	gcttaccatc	tggccccagt	gctgcaatga
4681	taccgcgaga	cccacgctca	ccggctccag	atztatcagc	aataaaccag	ccagccggaa
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4801	gccgggaagc	tagagtaagt	agttcgccag	ttaatagttt	gcgcaacggt	gttgccattg
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FIGURE 24C

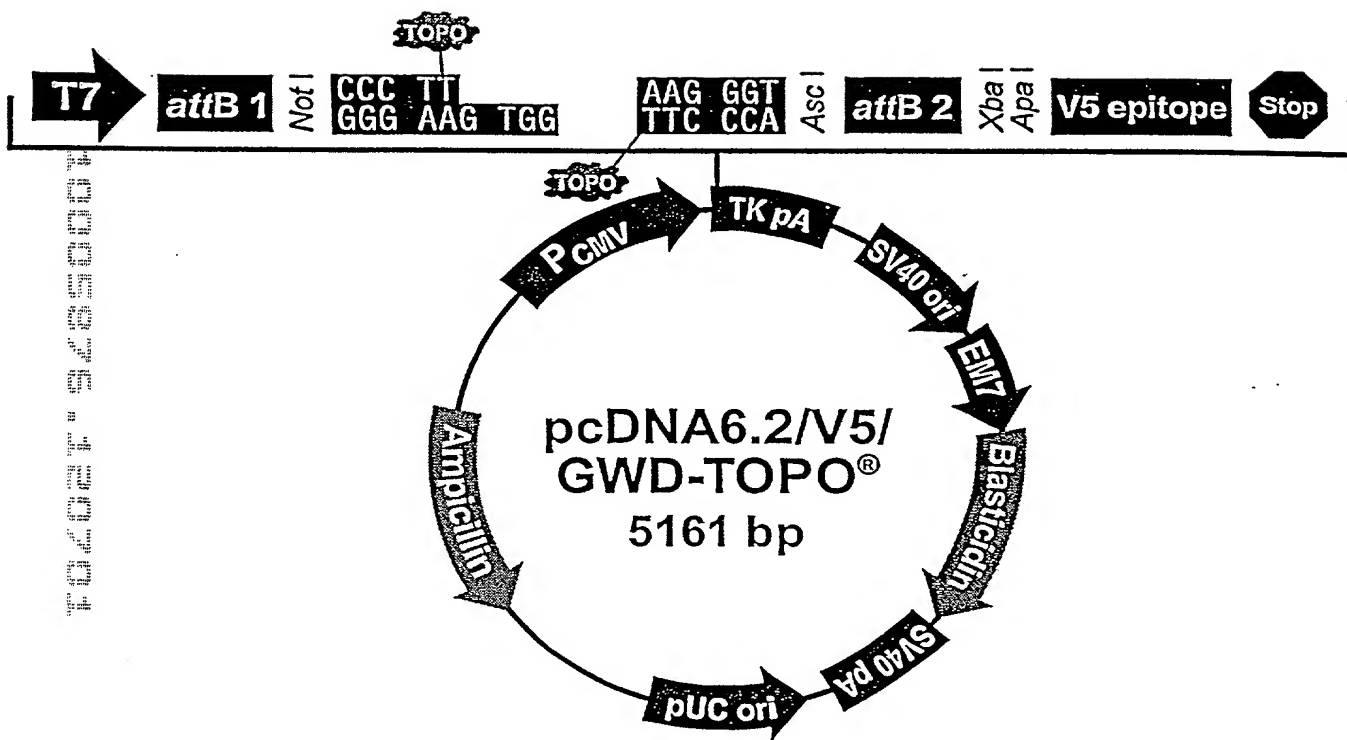


FIGURE 25A

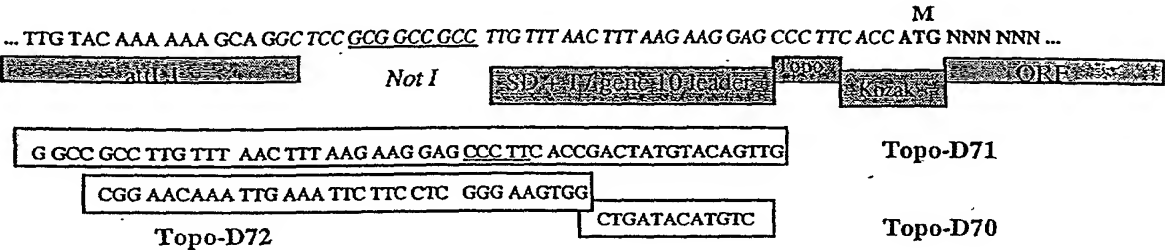
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841	ctgcttactg	gcttatcgaa	attaatacga	ctcactatag	ggagacccaa	gctggctagt
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1021	ccgcggttcg	aaggtaagcc	tatccctaac	cctctcctcg	gtctcgattc	tacgcgtacc
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FIGURE 25B

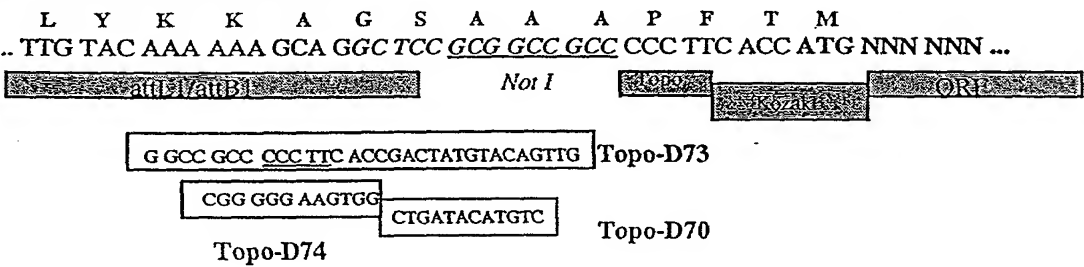
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FIGURE 25C

pENTR/SD-dTopo: 5' end



ENTR-dTopo and pcDNAGW-dTopo: 5' end



ENTR/SD-dTopo, pENTR-dTopo, and pcDNAGW-dTopo: 3' end

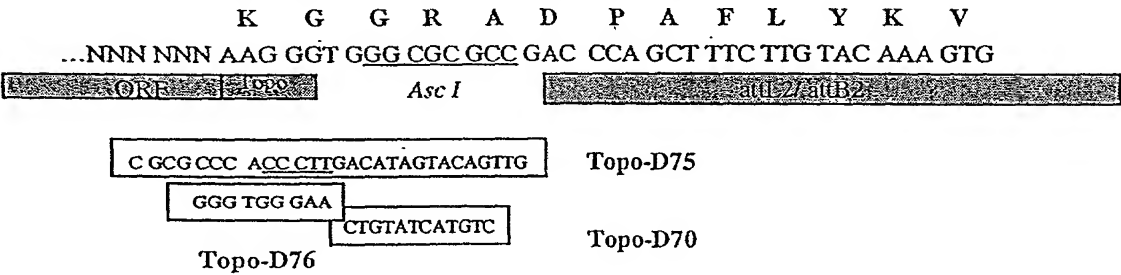


FIGURE 26

Species	Year	Sex	Age	Weight (g)	Length (mm)	Wing (mm)	Tail (mm)	Culmen (mm)	Gape (mm)	Bill (mm)	Foot (mm)	Toe (mm)	Middle toe (mm)	Claw (mm)	Max. gape (mm)	Max. bill (mm)	Max. foot (mm)	Max. toe (mm)	Max. middle toe (mm)	Max. claw (mm)
Red-tailed Tropicbird	1971	Male	Adult	100	110	55	45	15	10	25	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1972	Female	Adult	90	105	50	40	15	10	20	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1973	Male	Adult	110	115	60	50	15	10	25	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1974	Female	Adult	95	108	52	42	15	10	22	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1975	Male	Adult	105	112	58	48	15	10	24	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1976	Female	Adult	85	102	48	38	15	10	20	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1977	Male	Adult	115	118	62	52	15	10	26	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1978	Female	Adult	92	106	51	41	15	10	21	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1979	Male	Adult	108	114	59	49	15	10	25	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1980	Female	Adult	88	104	49	39	15	10	20	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1981	Male	Adult	112	116	61	51	15	10	25	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1982	Female	Adult	91	105	50	40	15	10	21	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1983	Male	Adult	107	113	58	48	15	10	24	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1984	Female	Adult	87	103	49	39	15	10	20	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1985	Male	Adult	111	115	59	49	15	10	24	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1986	Female	Adult	89	104	50	40	15	10	21	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1987	Male	Adult	109	114	59	49	15	10	25	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1988	Female	Adult	86	102	48	38	15	10	20	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1989	Male	Adult	113	117	61	51	15	10	26	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1990	Female	Adult	93	107	51	41	15	10	22	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1991	Male	Adult	110	116	60	50	15	10	25	15	10	10	10	10	10	10	10	10	10
Red-tailed Tropicbird	1992	Female	Adult	94	108	52	42	15	1											

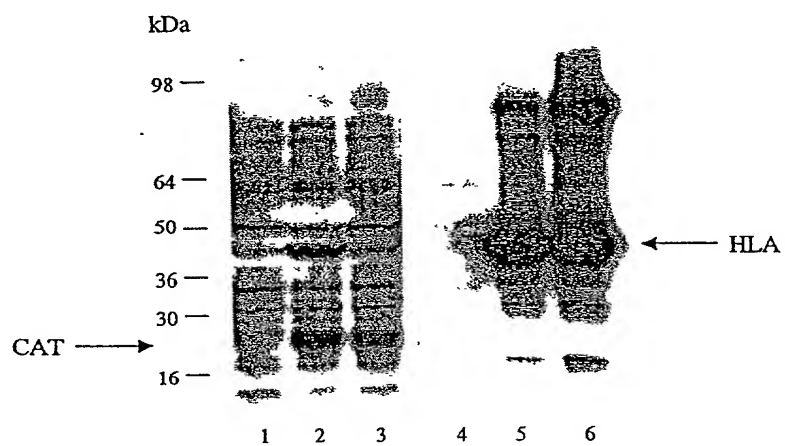


FIGURE 27

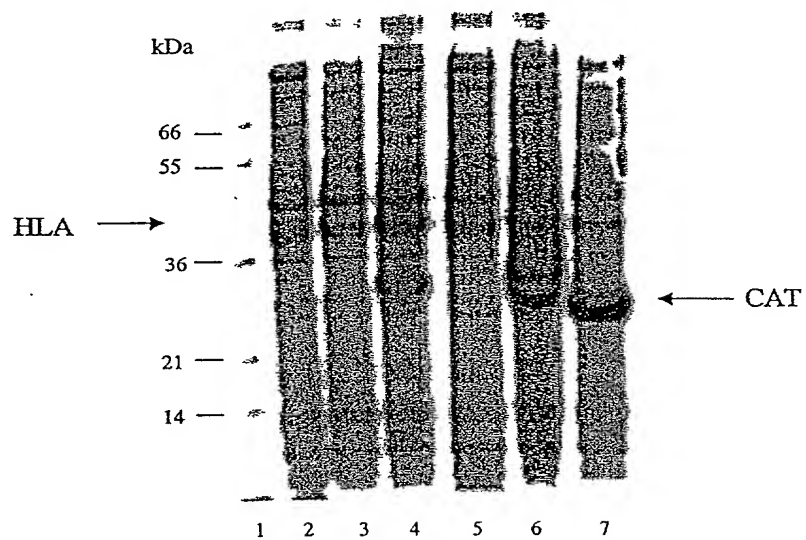


FIGURE 28

bioRxiv preprint doi: <https://doi.org/10.1101/000000>; this version posted January 1, 2014. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

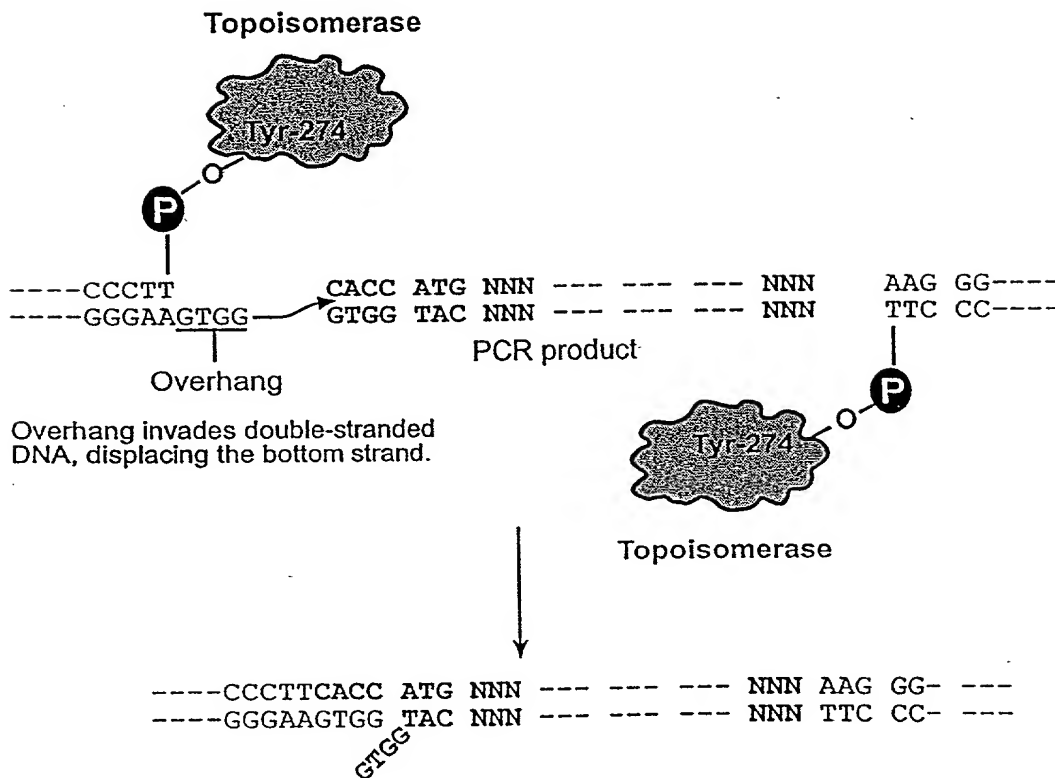
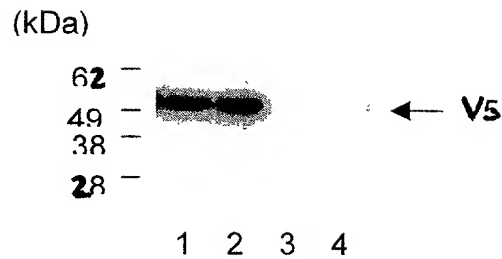


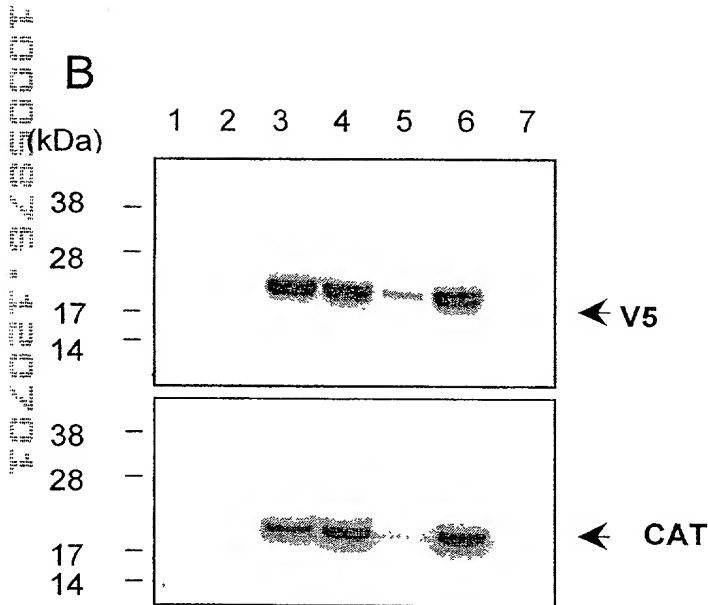
FIGURE 29

A



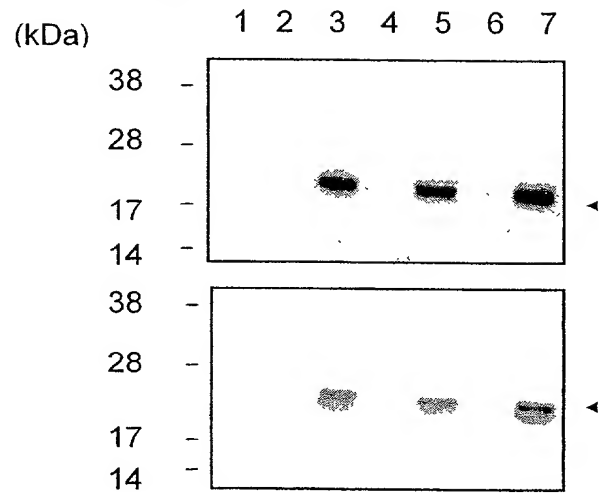
Lane 1: pCMVTetO/CAT/V5TKpA (without secondary PCR)+ Tet
 Lane 2: pCMVTetO/CAT/V5TKpA (with secondary PCR)+ Tet
 Lane 3: pCMVTetO/CAT/V5TKpA (with secondary PCR) - Tet
 Lane 4: pCMVTetO/CAT/V5TKpA (without secondary PCR)- Tet

B



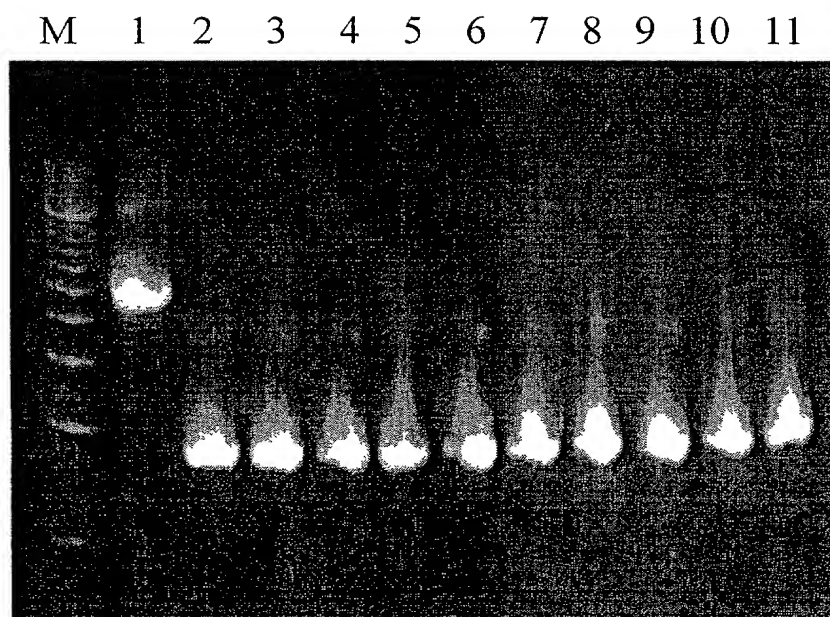
Lane 1: TRex-CHO Cells + Tet
 Lane 2: without secondary PCR (with purified CAT) - Tet
 Lane 3: without secondary PCR (with purified CAT)+ Tet
 Lane 4: without secondary PCR (with unpurified CAT) + Tet
 Lane 5: without secondary PCR (with unpurified CAT) - Tet
 Lane 6: with secondary PCR + Tet
 Lane 7: with secondary PCR -Tet

C



Lane 1: TRex-293 Cells + Tet
 Lane 2: without secondary PCR (with purified CAT) - Tet
 Lane 3: without secondary PCR (with purified CAT) + Tet
 Lane 4: without secondary PCR (with unpurified CAT) - Tet
 Lane 5: without secondary PCR (with unpurified CAT) + Tet
 Lane 6: with secondary PCR - Tet
 Lane 7: with secondary PCR + Tet

FIG. 30



Lane1: negative control; lanes 2-11: test clones; M: 500 bp marker

FIG. 31.

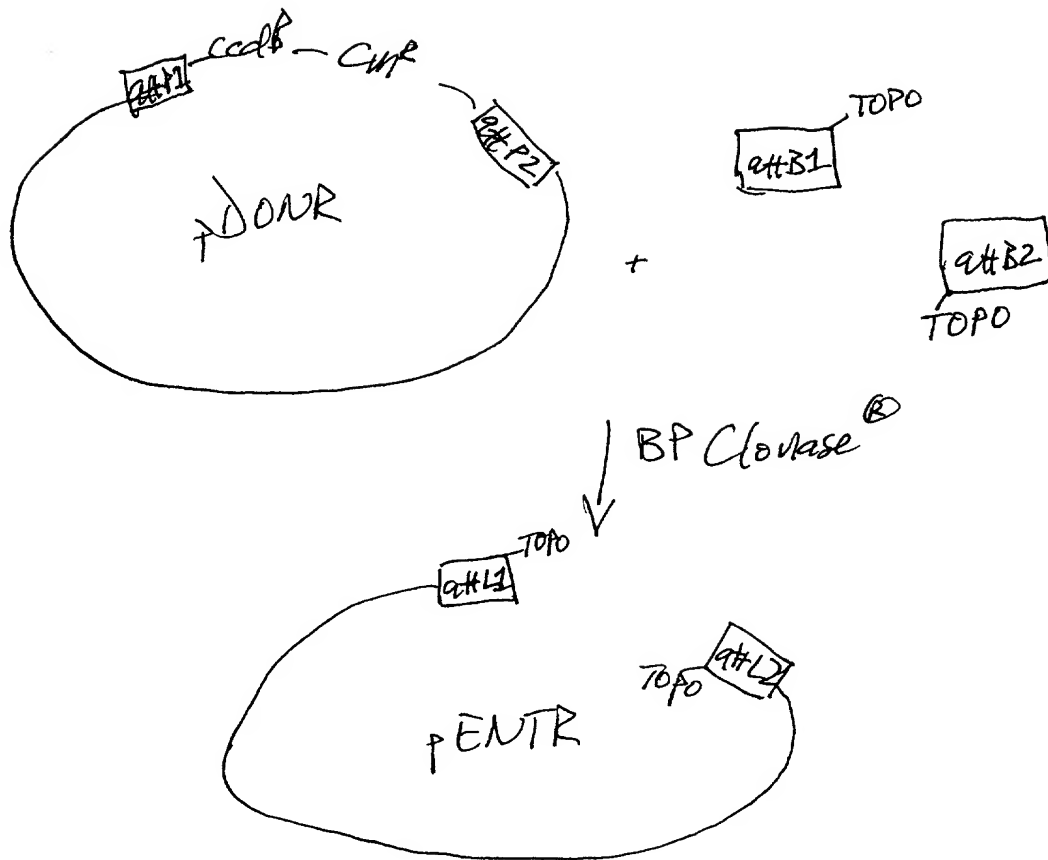
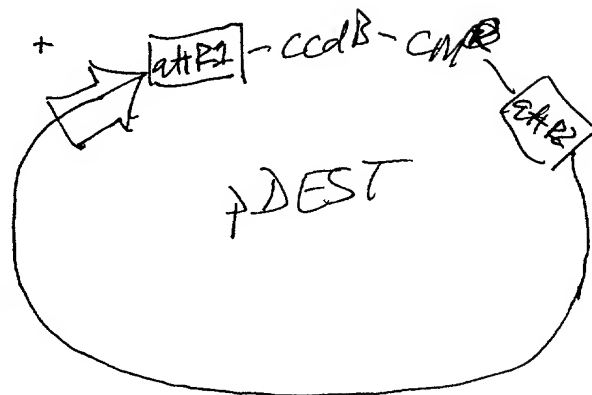
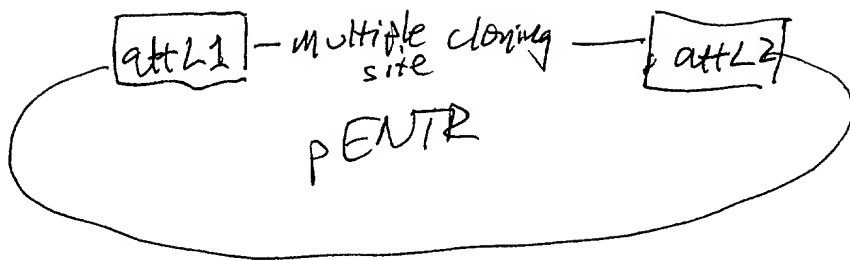


FIGURE 32



LR Clonase®



1. Cut with restriction enzymes;
2. Adapt with TOPO adapters;
3. Charge with TOPoisomerase.
4. Purify

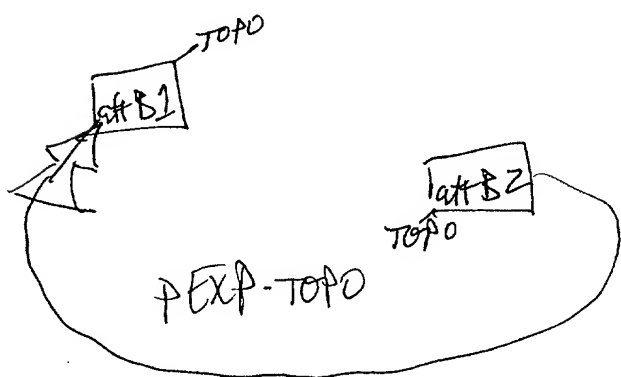


FIGURE 33

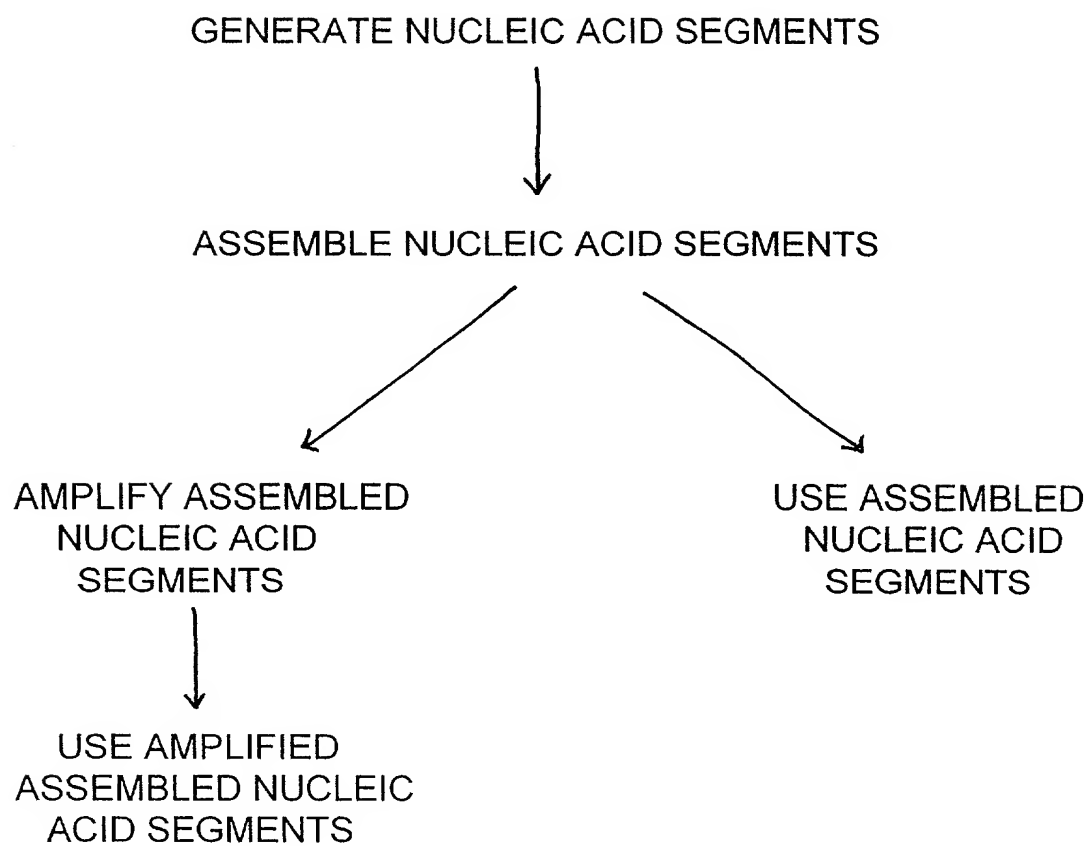


FIG. 34

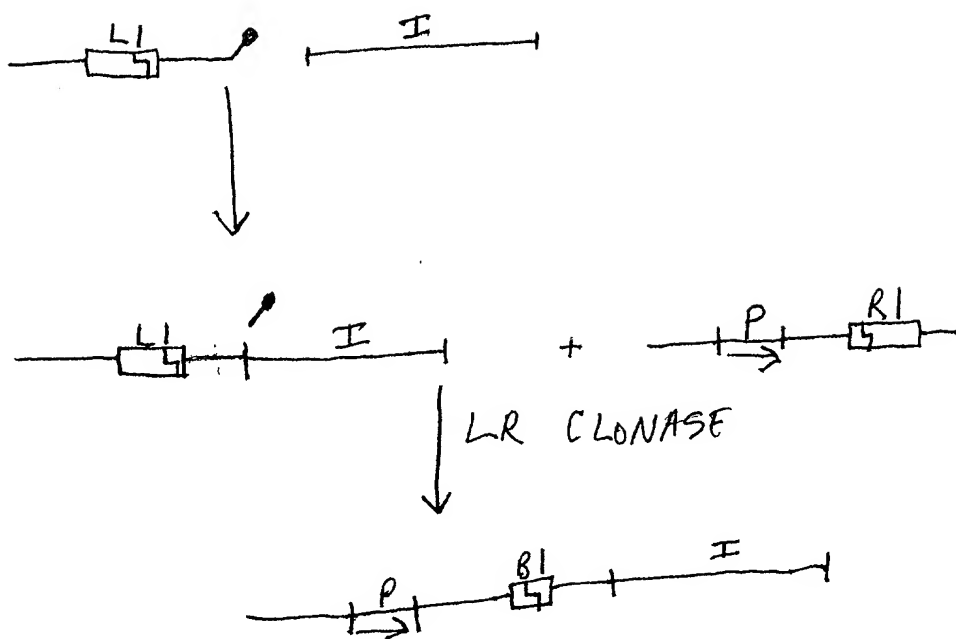


FIGURE 35

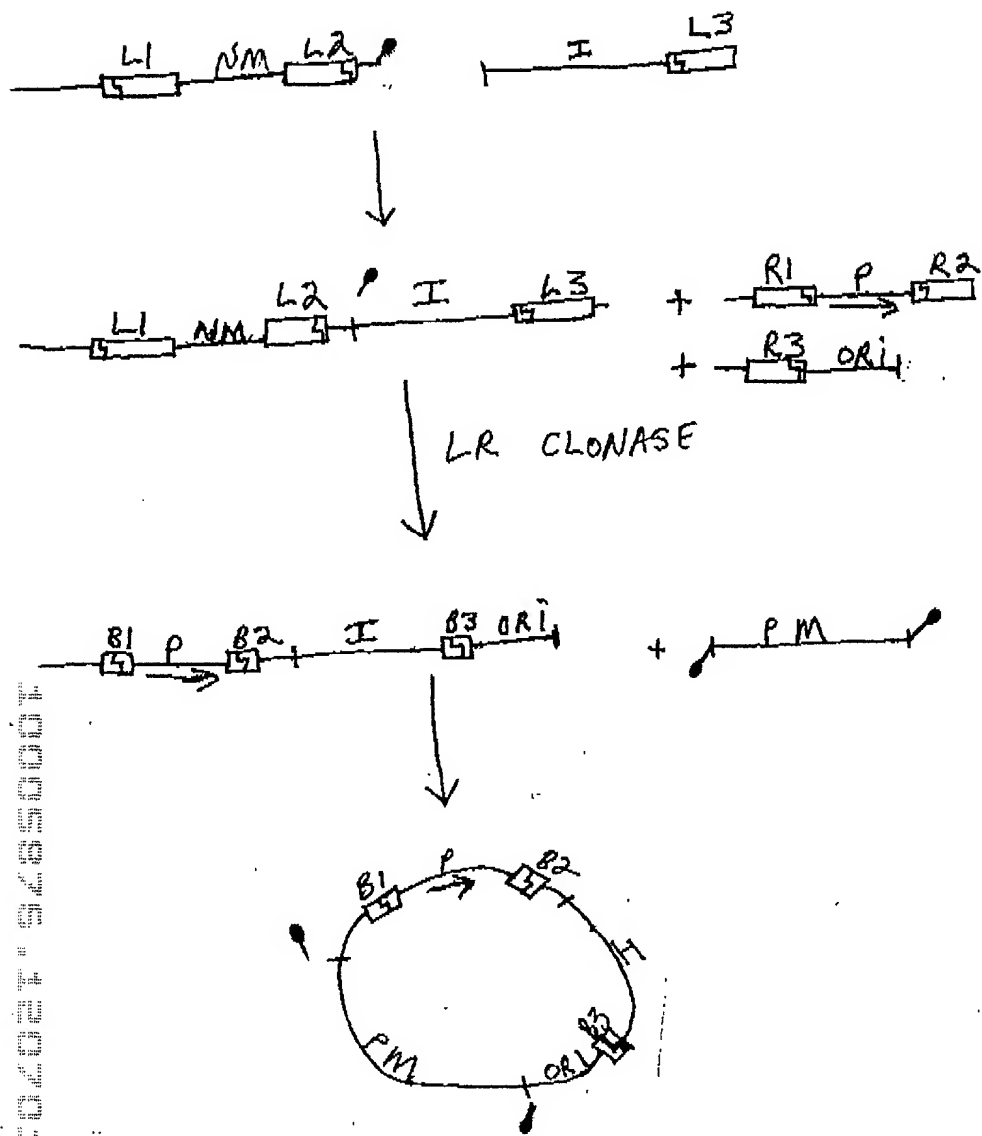


FIGURE 36

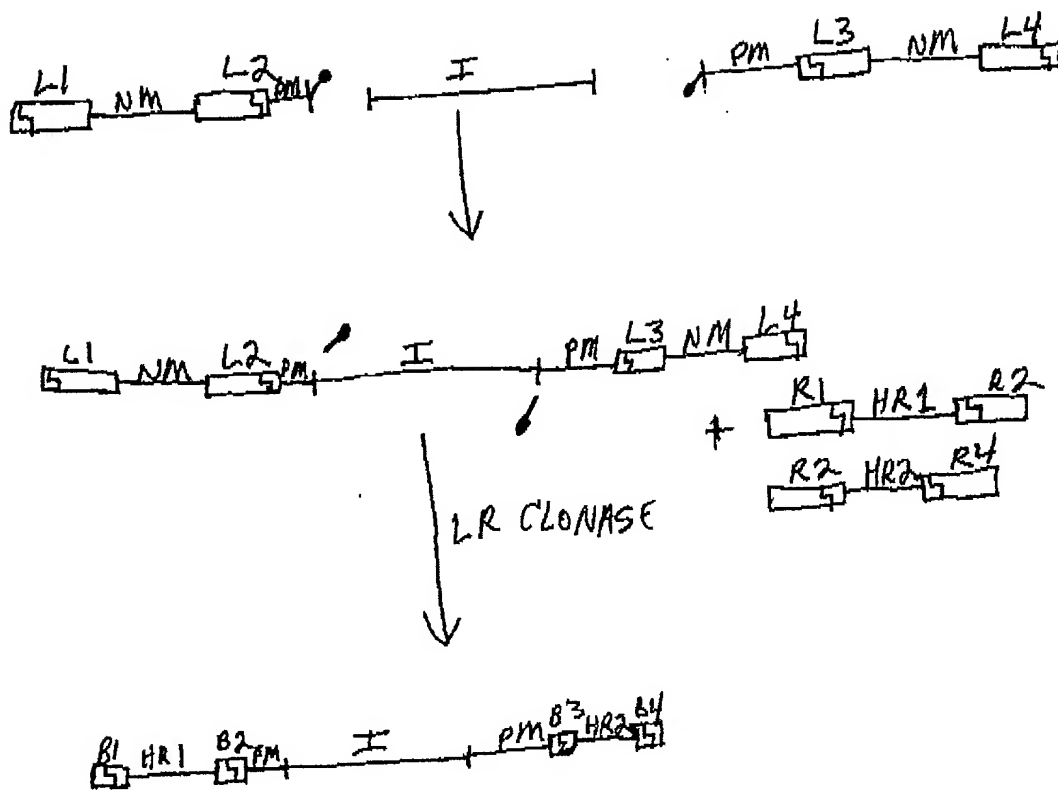


FIGURE 37

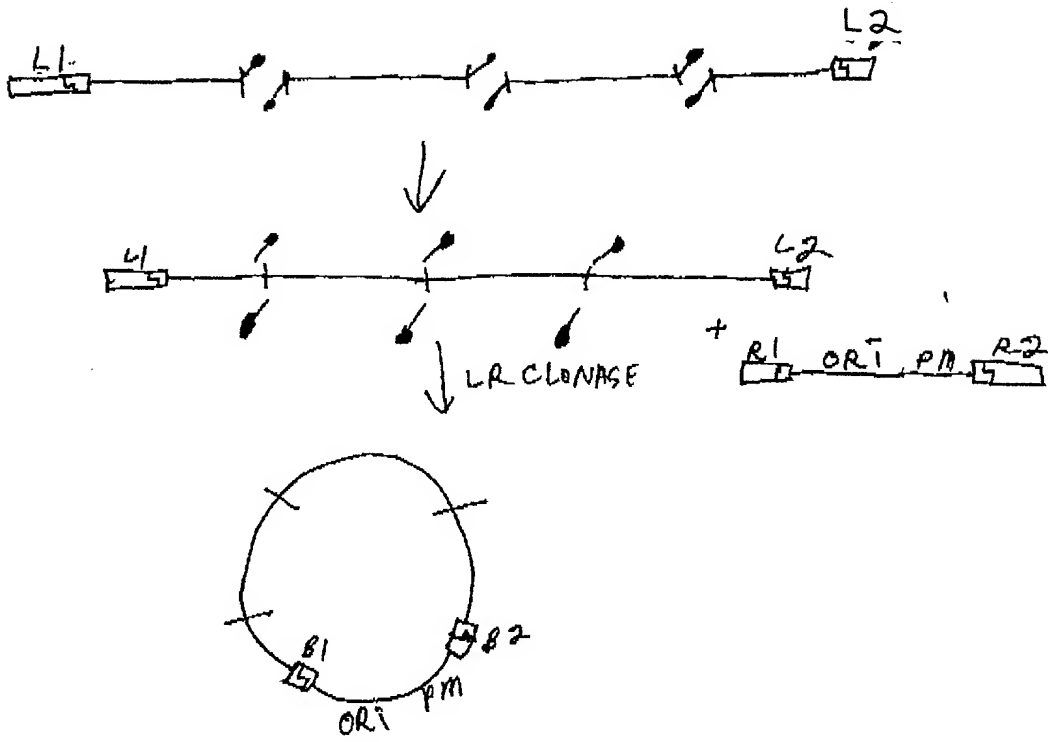


FIGURE 38

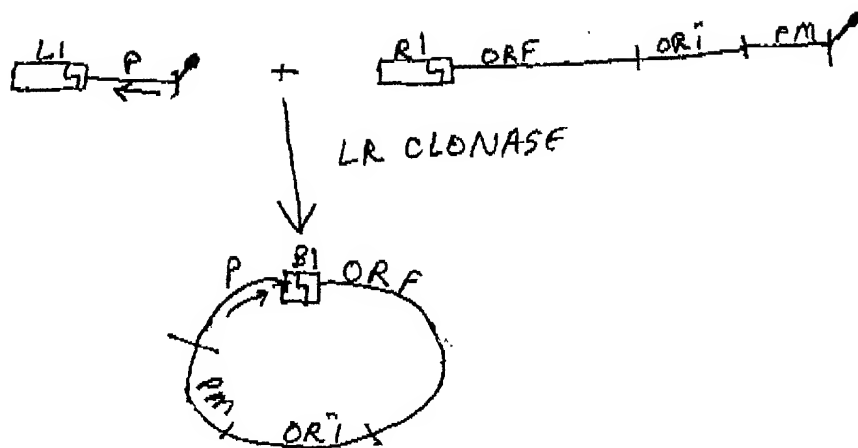
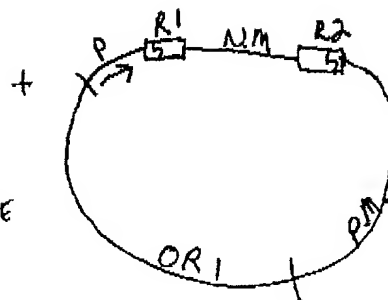
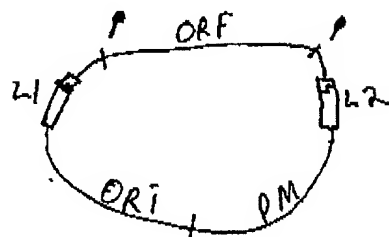


FIGURE 39



LR CLONASE

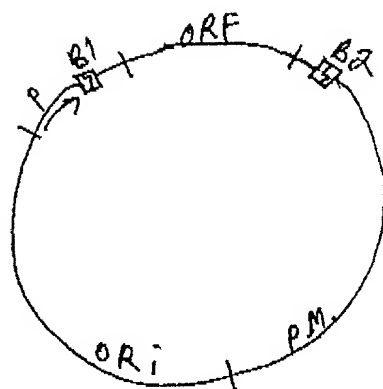


FIGURE 40